RNA INTERFERENCE MEDIATED TREATMENT OF ALZHEIMER'S DISEASE USING SHORT INTERFERING NUCLEIC ACID (siNA)

This application is a continuation-in-part of U.S. Patent Application No. 10/607,933, filed June 27, 2003, which is a continuation-in-part of U.S. Patent Application No. 09/930,423, filed August 15, 2001 and is also a continuation-in-part of International Patent Application No. PCT/US03/04710, filed February 18, 2003, which is a continuation-in-part of U.S. Patent Application No. 10/205,309, filed July 25, 2002. This application is also a continuation-in-part of International Patent Application No. PCT/US04/16390, filed May 24, 2004, which is a continuation-in-part of U.S. Patent Application No. 10/826,966, filed April 16, 2004, which is continuation-in-part of U.S. Patent Application No. 10/757,803, filed January 14, 2004, which is a continuation-in-part of U.S. Patent Application No. 10/720,448, filed November 24, 2003, which is a continuation-in-part of U.S. Patent Application No. 10/693,059, filed October 23, 2003, which is a continuation-in-part of U.S. Patent Application No. 10/444,853, filed May 23, 2003, which is a continuation-in-part of International Patent Application No. PCT/US03/05346, filed February 20, 2003, and a continuation-in-part of International Patent Application No. PCT/US03/05028, filed February 20, 2003, both of which claim the benefit of U.S. Provisional Application No. 60/358,580, filed February 20, 2002, U.S. Provisional Application No. 60/363,124, filed March 11, 2002, U.S. Provisional Application No. 60/386,782, filed June 6, 2002, U.S. Provisional Application No. 60/406,784, filed August 29, 2002, U.S. Provisional Application No. 60/408,378, filed September 5, 2002, U.S. Provisional Application No. 60/409,293, filed September 9, 2002, and U.S. Provisional Application No. 60/440,129, filed January 15, 2003. This application is also a continuation-in-part of International Patent Application No. PCT/US04/13456, filed April 30, 2004, which is a continuation of Patent Application No. 10/780,447, filed February 13, 2004, which is a continuation-in-part of US Patent Application No. 10/427,160, filed April 30, 2003, which is a continuation-in-part of International Patent Application No. PCT/US02/15876, filed May 17, 2002, which claims the benefit of U.S. Provisional Application No. 60/362,016, filed March 6, 2002, and U.S. Provisional Application No. 60/292,217, filed May 18, 2001. This application is also a continuation-in-part of U.S. Patent Application No. 10/727,780, filed December 3, 2003. This application also claims the benefit of U.S. Provisional Application No. 60/543,480, filed February 10, 2004. The instant application claims the benefit of all the

listed applications, which are hereby incorporated by reference herein in their entireties, including the drawings.

Field Of The Invention

The present invention relates to compounds, compositions, and methods for the study, diagnosis, and treatment of traits, diseases and conditions associated with Alzheimer's disease. The present invention is also directed to compounds, compositions, and methods relating to traits, diseases and conditions that respond to the modulation of expression and/or activity of genes involved in beta-secretase (BACE), amyloid precursor protein (APP), PIN-1, presenillin 1 (PS-1) and/or presenillin 2 (PS-2) gene expression pathways or other cellular processes that mediate the maintenance or development of such traits, diseases and conditions. Specifically, the invention relates to small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules capable of mediating RNA interference (RNAi) against beta-secretase (BACE), amyloid precursor protein (APP), PIN-1, presenillin 1 (PS-1) and/or presenillin 2 (PS-2) gene expression. Such small nucleic acid molecules are useful, for example, in providing compositions for treatment of traits, diseases and conditions that can respond to modulation of beta-secretase (BACE), amyloid precursor protein (APP), PIN-1, presenillin 1 (PS-1) and/or presenillin 2 (PS-2) gene expression in a subject, such as Alzheimer's disease or dementia.

Background Of The Invention

The following is a discussion of relevant art pertaining to RNAi. The discussion is provided only for understanding of the invention that follows. The summary is not an admission that any of the work described below is prior art to the claimed invention.

RNA interference refers to the process of sequence-specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs) (Zamore *et al.*, 2000, Cell, 101, 25-33; Fire *et al.*, 1998, *Nature*, 391, 806; Hamilton *et al.*, 1999, *Science*, 286, 950-951; Lin *et al.*, 1999, *Nature*, 402, 128-129; Sharp, 1999, Genes & Dev., 13:139-141; and Strauss, 1999, *Science*, 286, 886). The corresponding process in plants (Heifetz *et al.*, International PCT Publication No. WO 99/61631) is commonly referred to as post-transcriptional gene silencing or RNA silencing and is also referred to

as quelling in fungi. The process of post-transcriptional gene silencing is thought to be an evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes and is commonly shared by diverse flora and phyla (Fire *et al.*, 1999, *Trends Genet.*, 15, 358). Such protection from foreign gene expression may have evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or from the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA. The presence of dsRNA in cells triggers the RNAi response through a mechanism that has yet to be fully characterized. This mechanism appears to be different from other known mechanisms involving double stranded RNA-specific ribonucleases, such as the interferon response that results from dsRNA-mediated activation of protein kinase PKR and 2',5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L (see for example US Patent Nos. 6,107,094; 5,898,031; Clemens *et al.*, 1997, *J. Interferon & Cytokine Res.*, 17, 503-524; Adah *et al.*, 2001, *Curr. Med. Chem.*, 8, 1189).

The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as dicer (Bass, 2000, Cell, 101, 235; Zamore *et al.*, 2000, Cell, 101, 25-33; Hammond *et al.*, 2000, Nature, 404, 293). Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs) (Zamore *et al.*, 2000, Cell, 101, 25-33; Bass, 2000, Cell, 101, 235; Berstein *et al.*, 2001, *Nature*, 409, 363). Short interfering RNAs derived from dicer activity are typically about 21 to about 23 nucleotides in length and comprise about 19 base pair duplexes (Zamore *et al.*, 2000, Cell, 101, 25-33; Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188). Dicer has also been implicated in the excision of 21- and 22-nucleotide small temporal RNAs (stRNAs) from precursor RNA of conserved structure that are implicated in translational control (Hutvagner *et al.*, 2001, *Science*, 293, 834). The RNAi response also features an endonuclease complex, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded RNA having sequence complementary to the antisense strand of the siRNA duplex. Cleavage of the target RNA takes place in the middle of the region complementary to the antisense strand of the siRNA duplex (Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188).

RNAi has been studied in a variety of systems. Fire *et al.*, 1998, *Nature*, 391, 806, were the first to observe RNAi in *C. elegans*. Bahramian and Zarbl, 1999, *Molecular and Cellular Biology*, 19, 274-283 and Wianny and Goetz, 1999, *Nature Cell Biol.*, 2, 70, describe RNAi mediated by dsRNA in mammalian systems. Hammond *et al.*, 2000, *Nature*, 404, 293, describe RNAi in *Drosophila* cells transfected with dsRNA. Elbashir *et al.*, 2001, *Nature*, 411, 494 and Tuschl *et al.*, International PCT Publication No. WO 01/75164, describe RNAi induced by introduction of duplexes of synthetic 21-nucleotide RNAs in cultured mammalian cells including human embryonic kidney and HeLa cells. Recent work in Drosophila embryonic lysates (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877 and Tuschl *et al.*, International PCT Publication No. WO 01/75164) has revealed certain requirements for siRNA length, structure, chemical composition, and sequence that are essential to mediate efficient RNAi activity. These studies have shown that 21-nucleotide siRNA duplexes are most active when containing 3'-terminal dinucleotide overhangs. Furthermore, complete substitution of one or both siRNA strands with 2'-deoxy (2'-H) or 2'-O-methyl nucleotides abolishes RNAi activity, whereas substitution of the 3'-terminal siRNA overhang nucleotides with 2'-deoxy nucleotides (2'-H) was shown to be tolerated. Single mismatch sequences in the center of the siRNA duplex were also shown to abolish RNAi activity. In addition, these studies also indicate that the position of the cleavage site in the target RNA is defined by the 5'-end of the siRNA guide sequence rather than the 3'-end of the guide sequence (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877). Other studies have indicated that a 5'-phosphate on the target-complementary strand of a siRNA duplex is required for siRNA activity and that ATP is utilized to maintain the 5'-phosphate moiety on the siRNA (Nykanen *et al.*, 2001, *Cell*, 107, 309).

Studies have shown that replacing the 3'-terminal nucleotide overhanging segments of a 21-mer siRNA duplex having two-nucleotide 3'-overhangs with deoxyribonucleotides does not have an adverse effect on RNAi activity. Replacing up to four nucleotides on each end of the siRNA with deoxyribonucleotides has been reported to be well tolerated, whereas complete substitution with deoxyribonucleotides results in no RNAi activity (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877 and Tuschl *et al.*, International PCT Publication No. WO 01/75164). In addition, Elbashir *et al.*, *supra*, also report that substitution of siRNA with 2'-O-methyl nucleotides completely abolishes RNAi activity. Li *et al.*, International PCT Publication No. WO 00/44914, and Beach *et*

al., International PCT Publication No. WO 01/68836 preliminarily suggest that siRNA may include modifications to either the phosphate-sugar backbone or the nucleoside to include at least one of a nitrogen or sulfur heteroatom, however, neither application postulates to what extent such modifications would be tolerated in siRNA molecules, nor provides any further guidance or examples of such modified siRNA. Kreutzer *et al.*, Canadian Patent Application No. 2,359,180, also describe certain chemical modifications for use in dsRNA constructs in order to counteract activation of double-stranded RNA-dependent protein kinase PKR, specifically 2'-amino or 2'-O-methyl nucleotides, and nucleotides containing a 2'-O or 4'-C methylene bridge. However, Kreutzer *et al.* similarly fails to provide examples or guidance as to what extent these modifications would be tolerated in dsRNA molecules.

Parrish *et al.*, 2000, *Molecular Cell*, 6, 1077-1087, tested certain chemical modifications targeting the unc-22 gene in *C. elegans* using long (>25 nt) siRNA transcripts. The authors describe the introduction of thiophosphate residues into these siRNA transcripts by incorporating thiophosphate nucleotide analogs with T7 and T3 RNA polymerase and observed that RNAs with two phosphorothioate modified bases also had substantial decreases in effectiveness as RNAi. Further, Parrish *et al.* reported that phosphorothioate modification of more than two residues greatly destabilized the RNAs *in vitro* such that interference activities could not be assayed. *Id.* at 1081. The authors also tested certain modifications at the 2'-position of the nucleotide sugar in the long siRNA transcripts and found that substituting deoxynucleotides for ribonucleotides produced a substantial decrease in interference activity, especially in the case of Uridine to Thymidine and/or Cytidine to deoxy-Cytidine substitutions. *Id.* In addition, the authors tested certain base modifications, including substituting, in sense and antisense strands of the siRNA, 4-thiouracil, 5-bromouracil, 5-iodouracil, and 3-(aminoallyl)uracil for uracil, and inosine for guanosine. Whereas 4-thiouracil and 5-bromouracil substitution appeared to be tolerated, Parrish reported that inosine produced a substantial decrease in interference activity when incorporated in either strand. Parrish also reported that incorporation of 5-iodouracil and 3-(aminoallyl)uracil in the antisense strand resulted in a substantial decrease in RNAi activity as well.

The use of longer dsRNA has been described. For example, Beach *et al.*, International PCT Publication No. WO 01/68836, describes specific methods for

attenuating gene expression using endogenously-derived dsRNA. Tuschl *et al.*, International PCT Publication No. WO 01/75164, describe a *Drosophila in vitro* RNAi system and the use of specific siRNA molecules for certain functional genomic and certain therapeutic applications; although Tuschl, 2001, *Chem. Biochem.*, 2, 239-245, doubts that RNAi can be used to cure genetic diseases or viral infection due to the danger of activating interferon response. Li *et al.*, International PCT Publication No. WO 00/44914, describe the use of specific long (141 bp-488 bp) enzymatically synthesized or vector expressed dsRNAs for attenuating the expression of certain target genes. Zernicka-Goetz *et al.*, International PCT Publication No. WO 01/36646, describe certain methods for inhibiting the expression of particular genes in mammalian cells using certain long (550 bp-714 bp), enzymatically synthesized or vector expressed dsRNA molecules. Fire *et al.*, International PCT Publication No. WO 99/32619, describe particular methods for introducing certain long dsRNA molecules into cells for use in inhibiting gene expression in nematodes. Plaetinck *et al.*, International PCT Publication No. WO 00/01846, describe certain methods for identifying specific genes responsible for conferring a particular phenotype in a cell using specific long dsRNA molecules. Mello *et al.*, International PCT Publication No. WO 01/29058, describe the identification of specific genes involved in dsRNA-mediated RNAi. Pachuck *et al.*, International PCT Publication No. WO 00/63364, describe certain long (at least 200 nucleotide) dsRNA constructs. Deschamps Depaillette *et al.*, International PCT Publication No. WO 99/07409, describe specific compositions consisting of particular dsRNA molecules combined with certain anti-viral agents. Waterhouse *et al.*, International PCT Publication No. 99/53050 and 1998, *PNAS*, 95, 13959-13964, describe certain methods for decreasing the phenotypic expression of a nucleic acid in plant cells using certain dsRNAs. Driscoll *et al.*, International PCT Publication No. WO 01/49844, describe specific DNA expression constructs for use in facilitating gene silencing in targeted organisms.

Others have reported on various RNAi and gene-silencing systems. For example, Parrish *et al.*, 2000, *Molecular Cell*, 6, 1077-1087, describe specific chemically-modified dsRNA constructs targeting the unc-22 gene of *C. elegans*. Grossniklaus, International PCT Publication No. WO 01/38551, describes certain methods for regulating polycomb gene expression in plants using certain dsRNAs. Churikov *et al.*, International PCT

Publication No. WO 01/42443, describe certain methods for modifying genetic characteristics of an organism using certain dsRNAs. Cogoni *et al,*, International PCT Publication No. WO 01/53475, describe certain methods for isolating a Neurospora silencing gene and uses thereof. Reed *et al.*, International PCT Publication No. WO 01/68836, describe certain methods for gene silencing in plants. Honer *et al.*, International PCT Publication No. WO 01/70944, describe certain methods of drug screening using transgenic nematodes as Parkinson's Disease models using certain dsRNAs. Deak *et al.*, International PCT Publication No. WO 01/72774, describe certain *Drosophila*-derived gene products that may be related to RNAi in *Drosophila*. Arndt *et al.*, International PCT Publication No. WO 01/92513 describe certain methods for mediating gene suppression by using factors that enhance RNAi. Tuschl *et al.*, International PCT Publication No. WO 02/44321, describe certain synthetic siRNA constructs. Pachuk *et al.*, International PCT Publication No. WO 00/63364, and Satishchandran *et al.*, International PCT Publication No. WO 01/04313, describe certain methods and compositions for inhibiting the function of certain polynucleotide sequences using certain long (over 250 bp), vector expressed dsRNAs. Echeverri *et al.*, International PCT Publication No. WO 02/38805, describe certain *C. elegans* genes identified via RNAi. Kreutzer *et al.*, International PCT Publications Nos. WO 02/055692, WO 02/055693, and EP 1144623 B1 describes certain methods for inhibiting gene expression using dsRNA. Graham *et al.*, International PCT Publications Nos. WO 99/49029 and WO 01/70949, and AU 4037501 describe certain vector expressed siRNA molecules. Fire *et al.*, US 6,506,559, describe certain methods for inhibiting gene expression in vitro using certain long dsRNA (299 bp-1033 bp) constructs that mediate RNAi. Martinez *et al.*, 2002, *Cell*, 110, 563-574, describe certain single stranded siRNA constructs, including certain 5'-phosphorylated single stranded siRNAs that mediate RNA interference in Hela cells. Harborth *et al.*, 2003, Antisense & Nucleic Acid Drug Development, 13, 83-105, describe certain chemically and structurally modified siRNA molecules. Chiu and Rana, 2003, RNA, 9, 1034-1048, describe certain chemically and structurally modified siRNA molecules. Woolf *et al.*, International PCT Publication Nos. WO 03/064626 and WO 03/064625 describe certain chemically modified dsRNA constructs.

McSwiggen *et al.*, International PCT Publication No. WO 01/16312, describes nucleic acid mediated inhibition of BACE, PS-1, and PS-2 expression.

SUMMARY OF THE INVENTION

This invention relates to compounds, compositions, and methods useful for modulating the expression of genes associated with the maintenance or development of Alzheimer's disease and/or dementia, for example, beta-secretase (BACE), amyloid precursor protein (APP), PIN-1, presenillin 1 (PS-1) and/or presenillin 2 (PS-2) gene expression using short interfering nucleic acid (siNA) molecules. This invention also relates to compounds, compositions, and methods useful for modulating the expression and activity of other genes involved in pathways of BACE, APP, PIN-1, PS-1 and/or PS-2 gene expression and/or activity by RNA interference (RNAi) using small nucleic acid molecules. In particular, the instant invention features small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules and methods used to modulate the expression of BACE, APP, PIN-1, PS-1 and/or PS-2 genes or other genes associated with the maintenance or development of Alzheimer's disease and/or dementia.

A siNA of the invention can be unmodified or chemically-modified. A siNA of the instant invention can be chemically synthesized, expressed from a vector or enzymatically synthesized. The instant invention also features various chemically-modified synthetic short interfering nucleic acid (siNA) molecules capable of modulating BACE, APP, PIN-1, PS-1 and/or PS-2 gene expression or activity in cells by RNA interference (RNAi). The use of chemically-modified siNA improves various properties of native siNA molecules through increased resistance to nuclease degradation *in vivo* and/or through improved cellular uptake. Further, contrary to earlier published studies, siNA having multiple chemical modifications retains its RNAi activity. The siNA molecules of the instant invention provide useful reagents and methods for a variety of therapeutic, diagnostic, target validation, genomic discovery, genetic engineering, and pharmacogenomic applications.

In one embodiment, the invention features one or more siNA molecules and methods that independently or in combination modulate the expression of BACE, APP, PIN-1, PS-1 and/or PS-2 genes encoding proteins, such as proteins comprising BACE, APP, PIN-1, PS-1 and/or PS-2 associated with the maintenance and/or development of Alzheimer's disease and other neurodegenerative disorders or conditions such as dementia and stroke/cardiovascular accident (CVA), such as genes encoding sequences comprising those sequences referred to by GenBank Accession Nos. shown in **Table I**, referred to herein generally as BACE, APP, PIN-1, PS-1 and/or PS-2. The description below of the various aspects and embodiments of the invention is provided with reference to exemplary BACE gene referred to herein as BACE. However, the various aspects and embodiments are also directed to other BACE genes, such as BACE homolog genes, transcript variants and polymorphisms (e.g., single nucleotide polymorphism, (SNPs)) associated with certain BACE genes. As such, the various aspects and embodiments are also directed to other genes which express other BACE related proteins or other proteins associated with Alzheimer's disease, such as APP, PIN-1, PS-1 and/or PS-2, including mutant genes and splice variants thereof. The various aspects and embodiments are also directed to other genes that are involved in BACE, APP, PIN-1, PS-1 and/or PS-2 mediated pathways of signal transduction or gene expression that are involved, for example, in the progression, development, or maintenance of disease (e.g., Alzheimer's disease). These additional genes can be analyzed for target sites using the methods described for BACE genes herein. Thus, the modulation of other genes and the effects of such modulation of the other genes can be performed, determined, and measured as described herein.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a BACE gene, wherein said siNA molecule comprises about 18 to about 21 base pairs.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that directs cleavage of BACE RNA via RNA interference (RNAi), wherein the double stranded siNA molecule comprises a first and a second strand, each strand of the siNA molecule is about 18 to about 23 nucleotides in length, the first strand of the siNA molecule comprises nucleotide sequence having sufficient complementarity to the BACE RNA for the siNA molecule to direct cleavage of the

BACE RNA via RNA interference, and the second strand of said siNA molecule comprises nucleotide sequence that is complementary to the first strand.

In one embodiment, the invention features a chemically synthesized double stranded short interfering nucleic acid (siNA) molecule that directs cleavage of a BACE RNA via RNA interference (RNAi), wherein each strand of the siNA molecule is about 18 to about 23 nucleotides in length; and one strand of the siNA molecule comprises nucleotide sequence having sufficient complementarity to the BACE RNA for the siNA molecule to direct cleavage of the BACE RNA via RNA interference.

In one embodiment, the invention features a siNA molecule that down-regulates expression of a BACE gene, for example, wherein the BACE gene comprises BACE encoding sequence. In one embodiment, the invention features a siNA molecule that down-regulates expression of a BACE gene, for example, wherein the BACE gene comprises BACE non-coding sequence or regulatory elements involved in BACE gene expression.

In one embodiment, a siNA of the invention is used to inhibit the expression of BACE genes or a BACE gene family, wherein the genes or gene family sequences share sequence homology. Such homologous sequences can be identified as is known in the art, for example using sequence alignments. siNA molecules can be designed to target such homologous sequences, for example using perfectly complementary sequences or by incorporating non-canonical base pairs, for example mismatches and/or wobble base pairs, that can provide additional target sequences. In instances where mismatches are identified, non-canonical base pairs (for example, mismatches and/or wobble bases) can be used to generate siNA molecules that target more than one gene sequence. In a non-limiting example, non-canonical base pairs such as UU and CC base pairs are used to generate siNA molecules that are capable of targeting sequences for differing BACE targets that share sequence homology. As such, one advantage of using siNAs of the invention is that a single siNA can be designed to include nucleic acid sequence that is complementary to the nucleotide sequence that is conserved between the homologous genes. In this approach, a single siNA can be used to inhibit expression of more than one gene instead of using more than one siNA molecule to target the different genes.

In one embodiment, the invention features a siNA molecule having RNAi activity against BACE RNA, wherein the siNA molecule comprises a sequence complementary to any RNA having BACE encoding sequence, such as those sequences having GenBank Accession Nos. shown in **Table I**. In another embodiment, the invention features a siNA molecule having RNAi activity against BACE RNA, wherein the siNA molecule comprises a sequence complementary to an RNA having variant BACE encoding sequence, for example other mutant BCAE genes not shown in Table I but known in the art to be associated with the maintenance and/or development of Alzheimer's disease and/or dementia. Chemical modifications as shown in **Tables III and IV** or otherwise described herein can be applied to any siNA construct of the invention. In another embodiment, a siNA molecule of the invention includes a nucleotide sequence that can interact with nucleotide sequence of a BACE gene and thereby mediate silencing of BACE gene expression, for example, wherein the siNA mediates regulation of BACE gene expression by cellular processes that modulate the chromatin structure or methylation patterns of the BACE gene and prevent transcription of the BACE gene.

In one embodiment, siNA molecules of the invention are used to down regulate or inhibit the expression of BACE proteins arising from BACE haplotype polymorphisms that are associated with a disease or condition, (e.g., Alzheimer's disease and other neurodegenerative disorders or conditions such as dementia and stroke/cardiovascular accident (CVA)). Analysis of BACE genes, or BACE protein or RNA levels can be used to identify subjects with such polymorphisms or those subjects who are at risk of developing traits, conditions, or diseases described herein. These subjects are amenable to treatment, for example, treatment with siNA molecules of the invention and any other composition useful in treating diseases related to BACE gene expression. As such, analysis of BACE protein or RNA levels can be used to determine treatment type and the course of therapy in treating a subject. Monitoring of BACE protein or RNA levels can be used to predict treatment outcome and to determine the efficacy of compounds and compositions that modulate the level and/or activity of certain BACE proteins associated with a trait, condition, or disease.

In one embodiment of the invention a siNA molecule comprises an antisense strand comprising a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof encoding a BACE protein. The siNA further comprises a sense

strand, wherein said sense strand comprises a nucleotide sequence of a BACE gene or a portion thereof.

In another embodiment, a siNA molecule comprises an antisense region comprising a nucleotide sequence that is complementary to a nucleotide sequence encoding a BACE protein or a portion thereof. The siNA molecule further comprises a sense region, wherein said sense region comprises a nucleotide sequence of a BACE gene or a portion thereof.

In another embodiment, the invention features a siNA molecule comprising a nucleotide sequence in the antisense region of the siNA molecule that is complementary to a nucleotide sequence or portion of sequence of a BACE gene. In another embodiment, the invention features a siNA molecule comprising a region, for example, the antisense region of the siNA construct, complementary to a sequence comprising a BACE gene sequence or a portion thereof.

In one embodiment, the antisense region of BACE siNA constructs comprises a sequence complementary to sequence having any of SEQ ID NOs. 399-723, 1471-1478, 1591-1598, 1607-1614, 1623-1630, 1639-1646, 1655-1662, 1687, or 1689. In one embodiment, the antisense region of BACE constructs comprises sequence having any of SEQ ID NOs. 724-1048, 1599-1606, 1615-1622, 1631-1638, 1647-1654, 1663-1686, 1688, 1690, 1884, 1886, 1888, 1891, 1893, 1895, 1897, or 1900. In another embodiment, the sense region of BACE constructs comprises sequence having any of SEQ ID NOs. 399-723, 1471-1478, 1591-1598, 1607-1614, 1623-1630, 1639-1646, 1655-1662, 1687, 1689, 1883, 1885, 1887, 1889, 1890, 1892, 1894, 1896, 1898, or 1899.

In one embodiment, the antisense region of APP siNA constructs comprises a sequence complementary to sequence having any of SEQ ID NOs. 1-199, 1463-1470, 1495-1502, 1511-1518, 1527-1534, 1543-1550, or 1559-1566. In one embodiment, the antisense region of APP constructs comprises sequence having any of SEQ ID NOs. 200-398, 1503-1510, 1519-1526, 1535-1542, 1551-1558, 1567-1590, 1884, 1886, 1888, or 1891. In another embodiment, the sense region of APP constructs comprises sequence having any of SEQ ID NOs. 1-199, 1463-1470, 1495-1502, 1511-1518, 1527-1534, 1543-1550, 1559-1566, 1883, 1885, 1887, 1889, or 1890.

In one embodiment, the antisense region of PSEN1 siNA constructs comprises a sequence complementary to sequence having any of SEQ ID NOs. 1049-1131, 1479-1486, 1691-1698, 1707-1714, 1723-1730, 1739-1746, 1755-1762. In one embodiment, the antisense region of PSEN1 constructs comprises sequence having any of SEQ ID NOs. 1132-1214, 1699-1706, 1715-1722, 1731-1738, 1747-1754, 1763-1786, 1884, 1886, 1888, or 1891. In another embodiment, the sense region of PSEN1 constructs comprises sequence having any of SEQ ID NOs. 1049-1131, 1479-1486, 1691-1698, 1707-1714, 1723-1730, 1739-1746, 1755-1762, 1883, 1885, 1887, 1889, or 1890.

In one embodiment, the antisense region of PSEN2 siNA constructs comprises a sequence complementary to sequence having any of SEQ ID NOs. 1215-1338, 1487-1494, 1787-1794, 1803-1810, 1819-1826, 1835-1842, 1851-1858. In one embodiment, the antisense region of PSEN2 constructs comprises sequence having any of SEQ ID NOs. 1339-1462, 1795-1802, 1811-1818, 1827-1834, 1843-1850, 1859-1882, 1884, 1886, 1888, or 1891. In another embodiment, the sense region of PSEN2 constructs comprises sequence having any of SEQ ID NOs. SEQ ID NOs. 1215-1338, 1487-1494, 1787-1794, 1803-1810, 1819-1826, 1835-1842, 1851-1858, 1883, 1885, 1887, 1889, or 1890.

In one embodiment, a siNA molecule of the invention comprises any of SEQ ID NOs. 1-1900. The sequences shown in SEQ ID NOs: 1-1900 are not limiting. A siNA molecule of the invention can comprise any contiguous BACE sequence (e.g., about 18 to about 25, or about 18, 19, 20, 21, 22, 23, 24, or 25 contiguous BACE nucleotides).

In yet another embodiment, the invention features a siNA molecule comprising a sequence, for example, the antisense sequence of the siNA construct, complementary to a sequence or portion of sequence comprising sequence represented by GenBank Accession Nos. shown in **Table I**. Chemical modifications in **Tables III and IV** and described herein can be applied to any siNA construct of the invention.

In one embodiment of the invention a siNA molecule comprises an antisense strand having about 18 to about 29 (e.g., about 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) nucleotides, wherein the antisense strand is complementary to a RNA sequence encoding a BACE protein, and wherein said siNA further comprises a sense strand

having about 18 to about 29 (e.g., about 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) nucleotides, and wherein said sense strand and said antisense strand are distinct nucleotide sequences with at least about 18 complementary nucleotides.

In another embodiment of the invention a siNA molecule of the invention comprises an antisense region having about 18 to about 29 (e.g., about 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) nucleotides, wherein the antisense region is complementary to a RNA sequence encoding a BACE protein, and wherein said siNA further comprises a sense region having about 18 to about 29 (e.g., about 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) nucleotides, wherein said sense region and said antisense region comprise a linear molecule with at least about 19 complementary nucleotides.

In one embodiment, a siNA molecule of the invention has RNAi activity that modulates expression of RNA encoded by a BACE gene. Because BACE genes can share some degree of sequence homology with each other, siNA molecules can be designed to target a class of BACE genes or alternately specific BACE genes (e.g., polymorphic variants) by selecting sequences that are either shared amongst different BACE targets or alternatively that are unique for a specific BACE target. Therefore, in one embodiment, the siNA molecule can be designed to target conserved regions of BACE RNA sequences having homology among several BACE gene variants so as to target a class of BACE genes with one siNA molecule. Accordingly, in one embodiment, the siNA molecule of the invention modulates the expression of one or both BACE alleles in a subject. In another embodiment, the siNA molecule can be designed to target a sequence that is unique to a specific BACE RNA sequence (e.g., a single BACE allele or BACE single nucleotide polymorphism (SNP)) due to the high degree of specificity that the siNA molecule requires to mediate RNAi activity.

In one embodiment, nucleic acid molecules of the invention that act as mediators of the RNA interference gene silencing response are double-stranded nucleic acid molecules. In another embodiment, the siNA molecules of the invention consist of duplex nucleic acid molecules containing about 18 base pairs between oligonucleotides comprising about 18 to about 25 (*e.g.*, about 18, 19, 20, 21, 22, 23, 24, or 25) nucleotides. In yet another embodiment, siNA molecules of the invention comprise duplex nucleic acid molecules with overhanging ends of about about 1 to about 3 (*e.g.*,

about 1, 2, or 3) nucleotides, for example, about 21-nucleotide duplexes with about 18 base pairs and 3'-terminal mononucleotide, dinucleotide, or trinucleotide overhangs.

In one embodiment, the invention features one or more chemically-modified siNA constructs having specificity for BACE expressing nucleic acid molecules, such as RNA encoding a BACE protein. In one embodiment, the invention features a RNA based siNA molecule (e.g., a siNA comprising 2'-OH nucleotides) having specificity for BACE expressing nucleic acid molecules that includes one or more chemical modifications described herein. Non-limiting examples of such chemical modifications include without limitation phosphorothioate internucleotide linkages, 2'-deoxyribonucleotides, 2'-O-methyl ribonucleotides, 2'-deoxy-2'-fluoro ribonucleotides, "universal base" nucleotides, "acyclic" nucleotides, 5-C-methyl nucleotides, and terminal glyceryl and/or inverted deoxy abasic residue incorporation. These chemical modifications, when used in various siNA constructs, (e.g., RNA based siNA constructs), are shown to preserve RNAi activity in cells while at the same time, dramatically increasing the serum stability of these compounds. Furthermore, contrary to the data published by Parrish *et al., supra,* applicant demonstrates that multiple (greater than one) phosphorothioate substitutions are well-tolerated and confer substantial increases in serum stability for modified siNA constructs.

In one embodiment, a siNA molecule of the invention comprises modified nucleotides while maintaining the ability to mediate RNAi. The modified nucleotides can be used to improve *in vitro* or *in vivo* characteristics such as stability, activity, and/or bioavailability. For example, a siNA molecule of the invention can comprise modified nucleotides as a percentage of the total number of nucleotides present in the siNA molecule. As such, a siNA molecule of the invention can generally comprise about 5% to about 100% modified nucleotides (e.g., about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% modified nucleotides). The actual percentage of modified nucleotides present in a given siNA molecule will depend on the total number of nucleotides present in the siNA. If the siNA molecule is single stranded, the percent modification can be based upon the total number of nucleotides present in the single stranded siNA molecules. Likewise, if the siNA molecule is double stranded, the percent modification can be based upon the total

number of nucleotides present in the sense strand, antisense strand, or both the sense and antisense strands.

One aspect of the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a BACE gene. In one embodiment, the double stranded siNA molecule comprises one or more chemical modifications and each strand of the double-stranded siNA is about 21 nucleotides long. In one embodiment, the double-stranded siNA molecule does not contain any ribonucleotides. In another embodiment, the double-stranded siNA molecule comprises one or more ribonucleotides. In one embodiment, each strand of the double-stranded siNA molecule comprises about 18 to about 29 (e.g., about 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) nucleotides, wherein each strand comprises about 18 nucleotides that are complementary to the nucleotides of the other strand. In one embodiment, one of the strands of the double-stranded siNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of the BACE gene, and the second strand of the double-stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence of the BACE gene or a portion thereof.

In another embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a BACE gene comprising an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of the BACE gene or a portion thereof, and a sense region, wherein the sense region comprises a nucleotide sequence substantially similar to the nucleotide sequence of the BACE gene or a portion thereof. In one embodiment, the antisense region and the sense region each comprise about 18 to about 23 (e.g. about 18, 19, 20, 21, 22, or 23) nucleotides, wherein the antisense region comprises about 18 nucleotides that are complementary to nucleotides of the sense region.

In another embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a BACE gene comprising a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA

encoded by the BACE gene or a portion thereof and the sense region comprises a nucleotide sequence that is complementary to the antisense region.

In one embodiment, a siNA molecule of the invention comprises blunt ends, i.e., ends that do not include any overhanging nucleotides. For example, a siNA molecule comprising modifications described herein (e.g., comprising nucleotides having Formulae I-VII or siNA constructs comprising "Stab 00"-"Stab 25" (**Table IV**) or any combination thereof (see **Table IV**)) and/or any length described herein can comprise blunt ends or ends with no overhanging nucleotides.

In one embodiment, any siNA molecule of the invention can comprise one or more blunt ends, i.e. where a blunt end does not have any overhanging nucleotides. In one embodiment, the blunt ended siNA molecule has a number of base pairs equal to the number of nucleotides present in each strand of the siNA molecule. In another embodiment, the siNA molecule comprises one blunt end, for example wherein the 5'-end of the antisense strand and the 3'-end of the sense strand do not have any overhanging nucleotides. In another example, the siNA molecule comprises one blunt end, for example wherein the 3'-end of the antisense strand and the 5'-end of the sense strand do not have any overhanging nucleotides. In another example, a siNA molecule comprises two blunt ends, for example wherein the 3'-end of the antisense strand and the 5'-end of the sense strand as well as the 5'-end of the antisense strand and 3'-end of the sense strand do not have any overhanging nucleotides. A blunt ended siNA molecule can comprise, for example, from about 18 to about 30 nucleotides (e.g., about 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides). Other nucleotides present in a blunt ended siNA molecule can comprise, for example, mismatches, bulges, loops, or wobble base pairs to modulate the activity of the siNA molecule to mediate RNA interference.

By "blunt ends" is meant symmetric termini or termini of a double stranded siNA molecule having no overhanging nucleotides. The two strands of a double stranded siNA molecule align with each other without over-hanging nucleotides at the termini. For example, a blunt ended siNA construct comprises terminal nucleotides that are complementary between the sense and antisense regions of the siNA molecule.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a BACE gene, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule. The sense region can be connected to the antisense region via a linker molecule, such as a polynucleotide linker or a non-nucleotide linker.

In one embodiment, the invention features double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a BACE gene, wherein the siNA molecule comprises about 18 to about 21 base pairs, and wherein each strand of the siNA molecule comprises one or more chemical modifications. In another embodiment, one of the strands of the double-stranded siNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence of a BACE gene or a portion thereof, and the second strand of the double-stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence or a portion thereof of the BACE gene. In another embodiment, one of the strands of the double-stranded siNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence of a BACE gene or portion thereof, and the second strand of the double-stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence or portion thereof of the BACE gene. In another embodiment, each strand of the siNA molecule comprises about 18 to about 23 nucleotides, and each strand comprises at least about 18 nucleotides that are complementary to the nucleotides of the other strand. The BACE gene can comprise, for example, sequences referred to in **Table I.**

In one embodiment, a siNA molecule of the invention comprises no ribonucleotides. In another embodiment, a siNA molecule of the invention comprises ribonucleotides.

In one embodiment, a siNA molecule of the invention comprises an antisense region comprising a nucleotide sequence that is complementary to a nucleotide sequence of a BACE gene or a portion thereof, and the siNA further comprises a sense region comprising a nucleotide sequence substantially similar to the nucleotide sequence of the

BACE gene or a portion thereof. In another embodiment, the antisense region and the sense region each comprise about 18 to about 23 nucleotides and the antisense region comprises at least about 18 nucleotides that are complementary to nucleotides of the sense region. The BACE gene can comprise, for example, sequences referred to in **Table I.**

In one embodiment, a siNA molecule of the invention comprises a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA encoded by a BACE gene, or a portion thereof, and the sense region comprises a nucleotide sequence that is complementary to the antisense region. In one embodiment, the siNA molecule is assembled from two separate oligonucleotide fragments, wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule. In another embodiment, the sense region is connected to the antisense region via a linker molecule. In another embodiment, the sense region is connected to the antisense region via a linker molecule, such as a nucleotide or non-nucleotide linker. The BACE gene can comprise, for example, sequences referred in to **Table I.**

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a BACE gene comprising a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA encoded by the BACE gene or a portion thereof and the sense region comprises a nucleotide sequence that is complementary to the antisense region, and wherein the siNA molecule has one or more modified pyrimidine and/or purine nucleotides. In one embodiment, the pyrimidine nucleotides in the sense region are 2'-O-methyl pyrimidine nucleotides or 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-deoxy purine nucleotides. In another embodiment, the pyrimidine nucleotides in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-O-methyl purine nucleotides. In another embodiment, the pyrimidine nucleotides in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-deoxy purine nucleotides. In one embodiment, the pyrimidine nucleotides in the

antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the antisense region are 2'-O-methyl or 2'-deoxy purine nucleotides. In another embodiment of any of the above-described siNA molecules, any nucleotides present in a non-complementary region of the sense strand (e.g. overhang region) are 2'-deoxy nucleotides.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a BACE gene, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule, and wherein the fragment comprising the sense region includes a terminal cap moiety at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the fragment. In one embodiment, the terminal cap moiety is an inverted deoxy abasic moiety or glyceryl moiety. In one embodiment, each of the two fragments of the siNA molecule comprise about 21 nucleotides.

In one embodiment, the invention features a siNA molecule comprising at least one modified nucleotide, wherein the modified nucleotide is a 2'-deoxy-2'-fluoro nucleotide. The siNA can be, for example, of length between about 12 and about 36 nucleotides. In one embodiment, all pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides. In one embodiment, the modified nucleotides in the siNA include at least one 2'-deoxy-2'-fluoro cytidine or 2'-deoxy-2'-fluoro uridine nucleotide. In another embodiment, the modified nucleotides in the siNA include at least one 2'-fluoro cytidine and at least one 2'-deoxy-2'-fluoro uridine nucleotides. In one embodiment, all uridine nucleotides present in the siNA are 2'-deoxy-2'-fluoro uridine nucleotides. In one embodiment, all cytidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro cytidine nucleotides. In one embodiment, all adenosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro adenosine nucleotides. In one embodiment, all guanosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro guanosine nucleotides. The siNA can further comprise at least one modified internucleotidic linkage, such as phosphorothioate linkage. In one embodiment, the 2'-deoxy-2'-fluoronucleotides are present at specifically selected locations in the siNA that are sensitive to cleavage by ribonucleases, such as locations having pyrimidine nucleotides.

In one embodiment, the invention features a method of increasing the stability of a siNA molecule against cleavage by ribonucleases comprising introducing at least one modified nucleotide into the siNA molecule, wherein the modified nucleotide is a 2'-deoxy-2'-fluoro nucleotide. In one embodiment, all pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides. In one embodiment, the modified nucleotides in the siNA include at least one 2'-deoxy-2'-fluoro cytidine or 2'-deoxy-2'-fluoro uridine nucleotide. In another embodiment, the modified nucleotides in the siNA include at least one 2'-fluoro cytidine and at least one 2'-deoxy-2'-fluoro uridine nucleotides. In one embodiment, all uridine nucleotides present in the siNA are 2'-deoxy-2'-fluoro uridine nucleotides. In one embodiment, all cytidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro cytidine nucleotides. In one embodiment, all adenosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro adenosine nucleotides. In one embodiment, all guanosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro guanosine nucleotides. The siNA can further comprise at least one modified internucleotidic linkage, such as phosphorothioate linkage. In one embodiment, the 2'-deoxy-2'-fluoronucleotides are present at specifically selected locations in the siNA that are sensitive to cleavage by ribonucleases, such as locations having pyrimidine nucleotides.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a BACE gene comprising a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA encoded by the BACE gene or a portion thereof and the sense region comprises a nucleotide sequence that is complementary to the antisense region, and wherein the purine nucleotides present in the antisense region comprise 2'-deoxy- purine nucleotides. In an alternative embodiment, the purine nucleotides present in the antisense region comprise 2'-O-methyl purine nucleotides. In either of the above embodiments, the antisense region can comprise a phosphorothioate internucleotide linkage at the 3' end of the antisense region. Alternatively, in either of the above embodiments, the antisense region can comprise a glyceryl modification at the 3' end of the antisense region. In another embodiment of any of the above-described siNA molecules, any nucleotides

present in a non-complementary region of the antisense strand (e.g. overhang region) are 2'-deoxy nucleotides.

In one embodiment, the antisense region of a siNA molecule of the invention comprises sequence complementary to a portion of a BACE transcript having sequence unique to a particular BACE disease related allele, such as sequence comprising a single nucleotide polymorphism (SNP) associated with the disease specific allele. As such, the antisense region of a siNA molecule of the invention can comprise sequence complementary to sequences that are unique to a particular allele to provide specificity in mediating selective RNAi against the disease, condition, or trait related allele.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a BACE gene, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule. In another embodiment about 19 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule and wherein at least two 3' terminal nucleotides of each fragment of the siNA molecule are not base-paired to the nucleotides of the other fragment of the siNA molecule. In one embodiment, each of the two 3' terminal nucleotides of each fragment of the siNA molecule is a 2'-deoxy-pyrimidine nucleotide, such as a 2'-deoxy-thymidine. In another embodiment, all 21 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule. In another embodiment, about 19 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the BACE gene. In another embodiment, about 21 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the BACE gene. In any of the above embodiments, the 5'-end of the fragment comprising said antisense region can optionally includes a phosphate group.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits the expression of a BACE RNA sequence (e.g., wherein said target RNA sequence is encoded by a BACE gene involved in the BACE pathway), wherein the siNA molecule does not contain any ribonucleotides and

wherein each strand of the double-stranded siNA molecule is about 21 nucleotides long. Examples of non-ribonucleotide containing siNA constructs are combinations of stabilization chemistries shown in **Table IV** in any combination of Sense/Antisense chemistries, such as Stab 7/8, Stab 7/11, Stab 8/8, Stab 18/8, Stab 18/11, Stab 12/13, Stab 7/13, Stab 18/13, Stab 7/19, Stab 8/19, Stab 18/19, Stab 7/20, Stab 8/20, or Stab 18/20.

In one embodiment, the invention features a chemically synthesized double stranded RNA molecule that directs cleavage of a BACE RNA via RNA interference, wherein each strand of said RNA molecule is about 21 to about 23 nucleotides in length; one strand of the RNA molecule comprises nucleotide sequence having sufficient complementarity to the BACE RNA for the RNA molecule to direct cleavage of the BACE RNA via RNA interference; and wherein at least one strand of the RNA molecule comprises one or more chemically modified nucleotides described herein, such as deoxynucleotides, 2'-O-methyl nucleotides, 2'-deoxy-2'-fluoro nucloetides, 2'-O-methoxyethyl nucleotides etc.

In one embodiment, the invention features a medicament comprising a siNA molecule of the invention.

In one embodiment, the invention features an active ingredient comprising a siNA molecule of the invention.

In one embodiment, the invention features the use of a double-stranded short interfering nucleic acid (siNA) molecule to down-regulate expression of a BACE gene, wherein the siNA molecule comprises one or more chemical modifications and each strand of the double-stranded siNA is about 18 to about 28 or more (e.g., about 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 or more) nucleotides long.

In one embodiment, the invention features the use of a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BACE gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BACE RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense

strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BACE gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BACE RNA or a portion thereof, wherein the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BACE gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BACE RNA that encodes a protein or portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification. In one embodiment, each strand of the siNA molecule comprises about 18 to about 29 or more (e.g., about 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29 or more) nucleotides, wherein each strand comprises at least about 18 nucleotides that are complementary to the nucleotides of the other strand. In one embodiment, the siNA molecule is assembled from two oligonucleotide fragments, wherein one fragment comprises the nucleotide sequence of the antisense strand of the siNA molecule and a second fragment comprises nucleotide sequence of the sense region of the siNA molecule. In one embodiment, the sense strand is connected to the antisense strand via a linker molecule, such as a polynucleotide linker or a non-nucleotide linker. In a further embodiment, the pyrimidine nucleotides present in the sense strand are 2'-deoxy-2'fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-deoxy purine nucleotides. In another embodiment, the pyrimidine nucleotides present in the sense strand are 2'-deoxy-2'fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-O-methyl purine nucleotides. In still another embodiment, the pyrimidine

nucleotides present in the antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and any purine nucleotides present in the antisense strand are 2'-deoxy purine nucleotides. In another embodiment, the antisense strand comprises one or more 2'-deoxy-2'-fluoro pyrimidine nucleotides and one or more 2'-O-methyl purine nucleotides. In another embodiment, the pyrimidine nucleotides present in the antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and any purine nucleotides present in the antisense strand are 2'-O-methyl purine nucleotides. In a further embodiment the sense strand comprises a 3'-end and a 5'-end, wherein a terminal cap moiety (e.g., an inverted deoxy abasic moiety or inverted deoxy nucleotide moiety such as inverted thymidine) is present at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the sense strand. In another embodiment, the antisense strand comprises a phosphorothioate internucleotide linkage at the 3' end of the antisense strand. In another embodiment, the antisense strand comprises a glyceryl modification at the 3' end. In another embodiment, the 5'-end of the antisense strand optionally includes a phosphate group.

In any of the above-described embodiments of a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BACE gene, wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, each of the two strands of the siNA molecule can comprise about 21 nucleotides. In one embodiment, about 21 nucleotides of each strand of the siNA molecule are base-paired to the complementary nucleotides of the other strand of the siNA molecule. In another embodiment, about 19 nucleotides of each strand of the siNA molecule are base-paired to the complementary nucleotides of the other strand of the siNA molecule, wherein at least two 3' terminal nucleotides of each strand of the siNA molecule are not base-paired to the nucleotides of the other strand of the siNA molecule. In another embodiment, each of the two 3' terminal nucleotides of each fragment of the siNA molecule is a 2'-deoxy-pyrimidine, such as 2'-deoxy-thymidine. In one embodiment, each strand of the siNA molecule is base-paired to the complementary nucleotides of the other strand of the siNA molecule. In one embodiment, about 19 nucleotides of the antisense strand are base-paired to the nucleotide sequence of the BACE RNA or a portion thereof. In one embodiment, about 21 nucleotides of the antisense strand are base-paired to the nucleotide sequence of the BACE RNA or a portion thereof.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BACE gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BACE RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the 5'-end of the antisense strand optionally includes a phosphate group.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BACE gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BACE RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the nucleotide sequence or a portion thereof of the antisense strand is complementary to a nucleotide sequence of the untranslated region or a portion thereof of the BACE RNA.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BACE gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BACE RNA or a portion thereof, wherein the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand, wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the nucleotide sequence of the antisense strand is complementary to a nucleotide sequence of the BACE RNA or a portion thereof that is present in the BACE RNA.

In one embodiment, the invention features a composition comprising a siNA molecule of the invention in a pharmaceutically acceptable carrier or diluent.

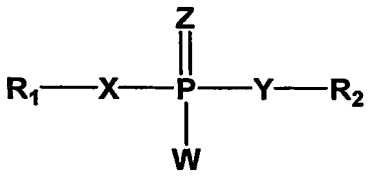
In a non-limiting example, the introduction of chemically-modified nucleotides into nucleic acid molecules provides a powerful tool in overcoming potential limitations of *in vivo* stability and bioavailability inherent to native RNA molecules that are delivered exogenously. For example, the use of chemically-modified nucleic acid molecules can enable a lower dose of a particular nucleic acid molecule for a given therapeutic effect since chemically-modified nucleic acid molecules tend to have a longer half-life in serum. Furthermore, certain chemical modifications can improve the bioavailability of nucleic acid molecules by targeting particular cells or tissues and/or improving cellular uptake of the nucleic acid molecule. Therefore, even if the activity of a chemically-modified nucleic acid molecule is reduced as compared to a native nucleic acid molecule, for example, when compared to an all-RNA nucleic acid molecule, the overall activity of the modified nucleic acid molecule can be greater than that of the native molecule due to improved stability and/or delivery of the molecule. Unlike native unmodified siNA, chemically-modified siNA can also minimize the possibility of activating interferon activity in humans.

In any of the embodiments of siNA molecules described herein, the antisense region of a siNA molecule of the invention can comprise a phosphorothioate internucleotide linkage at the 3'-end of said antisense region. In any of the embodiments of siNA molecules described herein, the antisense region can comprise about one to about five phosphorothioate internucleotide linkages at the 5'-end of said antisense region. In any of the embodiments of siNA molecules described herein, the 3'-terminal nucleotide overhangs of a siNA molecule of the invention can comprise ribonucleotides or deoxyribonucleotides that are chemically-modified at a nucleic acid sugar, base, or backbone. In any of the embodiments of siNA molecules described herein, the 3'-terminal nucleotide overhangs can comprise one or more universal base ribonucleotides. In any of the embodiments of siNA molecules described herein, the 3'-terminal nucleotide overhangs can comprise one or more acyclic nucleotides.

One embodiment of the invention provides an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the invention in a manner that allows expression of the nucleic acid molecule. Another embodiment of the invention provides a mammalian cell comprising such an expression vector. The mammalian cell can be a human cell. The siNA molecule of the expression vector can

comprise a sense region and an antisense region. The antisense region can comprise sequence complementary to a RNA or DNA sequence encoding BACE and the sense region can comprise sequence complementary to the antisense region. The siNA molecule can comprise two distinct strands having complementary sense and antisense regions. The siNA molecule can comprise a single strand having complementary sense and antisense regions.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against BACE inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides comprising a backbone modified internucleotide linkage having Formula I:

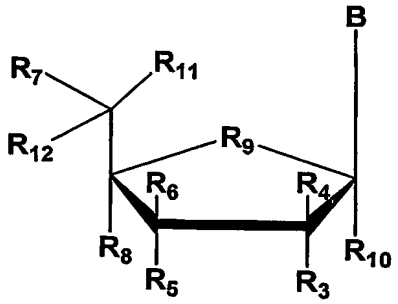


wherein each R1 and R2 is independently any nucleotide, non-nucleotide, or polynucleotide which can be naturally-occurring or chemically-modified, each X and Y is independently O, S, N, alkyl, or substituted alkyl, each Z and W is independently O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, aralkyl, or acetyl and wherein W, X, Y, and Z are optionally not all O. In another embodiment, a backbone modification of the invention comprises a phosphonoacetate and/or thiophosphonoacetate internucleotide linkage (see for example Sheehan et al., 2003, Nucleic Acids Research, 31, 4109-4118).

The chemically-modified internucleotide linkages having Formula I, for example, wherein any Z, W, X, and/or Y independently comprises a sulphur atom, can be present in one or both oligonucleotide strands of the siNA duplex, for example, in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) chemically-modified internucleotide linkages having Formula I at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified internucleotide linkages having

Formula I at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) pyrimidine nucleotides with chemically-modified internucleotide linkages having Formula I in the sense strand, the antisense strand, or both strands. In yet another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) purine nucleotides with chemically-modified internucleotide linkages having Formula I in the sense strand, the antisense strand, or both strands. In another embodiment, a siNA molecule of the invention having internucleotide linkage(s) of Formula I also comprises a chemically-modified nucleotide or non-nucleotide having any of Formulae I-VII.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against BACE inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides or non-nucleotides having Formula II:

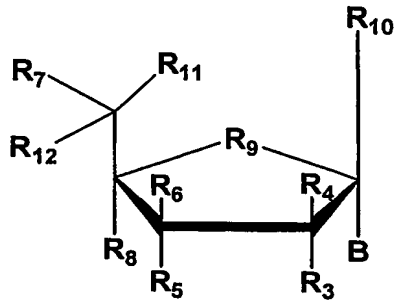


wherein each R3, R4, R5, R6, R7, R8, R10, R11 and R12 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalklylamino, substituted silyl, or group having Formula I or II; R9 is O, S, CH2, S=O, CHF, or CF2, and B is a nucleosidic base such as adenine, guanine, uracil, cytosine, thymine, 2-aminoadenosine, 5-methylcytosine, 2,6-diaminopurine, or any other non-naturally

occurring base that can be complementary or non-complementary to target RNA or a non-nucleosidic base such as phenyl, naphthyl, 3-nitropyrrole, 5-nitroindole, nebularine, pyridone, pyridinone, or any other non-naturally occurring universal base that can be complementary or non-complementary to target RNA.

The chemically-modified nucleotide or non-nucleotide of Formula II can be present in one or both oligonucleotide strands of the siNA duplex, for example in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more chemically-modified nucleotide or non-nucleotide of Formula II at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified nucleotides or non-nucleotides of Formula II at the 5'-end of the sense strand, the antisense strand, or both strands. In anther non-limiting example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified nucleotides or non-nucleotides of Formula II at the 3'-end of the sense strand, the antisense strand, or both strands.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against BACE inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides or non-nucleotides having Formula III:



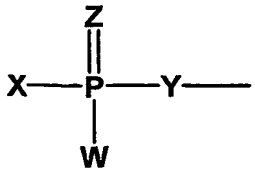
wherein each R3, R4, R5, R6, R7, R8, R10, R11 and R12 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH,

O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalklylamino, substituted silyl, or group having Formula I or II; R9 is O, S, CH2, S=O, CHF, or CF2, and B is a nucleosidic base such as adenine, guanine, uracil, cytosine, thymine, 2-aminoadenosine, 5-methylcytosine, 2,6-diaminopurine, or any other non-naturally occurring base that can be employed to be complementary or non-complementary to target RNA or a non-nucleosidic base such as phenyl, naphthyl, 3-nitropyrrole, 5-nitroindole, nebularine, pyridone, pyridinone, or any other non-naturally occurring universal base that can be complementary or non-complementary to target RNA.

The chemically-modified nucleotide or non-nucleotide of Formula III can be present in one or both oligonucleotide strands of the siNA duplex, for example, in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more chemically-modified nucleotide or non-nucleotide of Formula III at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified nucleotide(s) or non-nucleotide(s) of Formula III at the 5'-end of the sense strand, the antisense strand, or both strands. In anther non-limiting example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified nucleotide or non-nucleotide of Formula III at the 3'-end of the sense strand, the antisense strand, or both strands.

In another embodiment, a siNA molecule of the invention comprises a nucleotide having Formula II or III, wherein the nucleotide having Formula II or III is in an inverted configuration. For example, the nucleotide having Formula II or III is connected to the siNA construct in a 3'-3', 3'-2', 2'-3', or 5'-5' configuration, such as at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both siNA strands.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against BACE inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises a 5'-terminal phosphate group having Formula IV:



wherein each X and Y is independently O, S, N, alkyl, substituted alkyl, or alkylhalo; wherein each Z and W is independently O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, aralkyl, alkylhalo, or acetyl; and wherein W, X, Y and Z are not all O.

In one embodiment, the invention features a siNA molecule having a 5'-terminal phosphate group having Formula IV on the target-complementary strand, for example, a strand complementary to a target RNA, wherein the siNA molecule comprises an all RNA siNA molecule. In another embodiment, the invention features a siNA molecule having a 5'-terminal phosphate group having Formula IV on the target-complementary strand wherein the siNA molecule also comprises about 1 to about 3 (*e.g.*, about 1, 2, or 3) nucleotide 3'-terminal nucleotide overhangs having about 1 to about 4 (*e.g.*, about 1, 2, 3, or 4) deoxyribonucleotides on the 3'-end of one or both strands. In another embodiment, a 5'-terminal phosphate group having Formula IV is present on the target-complementary strand of a siNA molecule of the invention, for example a siNA molecule having chemical modifications having any of Formulae I-VII.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against BACE inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more phosphorothioate internucleotide linkages. For example, in a non-limiting example, the invention features a chemically-modified short interfering nucleic acid (siNA) having about 1, 2, 3, 4, 5, 6, 7, 8 or more phosphorothioate internucleotide linkages in one siNA strand. In yet another embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) individually having about 1, 2, 3, 4, 5, 6, 7, 8 or more phosphorothioate internucleotide linkages in both siNA strands. The phosphorothioate internucleotide linkages can be present in one or both oligonucleotide strands of the siNA duplex, for example in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more phosphorothioate internucleotide linkages at the 3'-end, the 5'-end, or both of the 3'- and

5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) consecutive phosphorothioate internucleotide linkages at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) pyrimidine phosphorothioate internucleotide linkages in the sense strand, the antisense strand, or both strands. In yet another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) purine phosphorothioate internucleotide linkages in the sense strand, the antisense strand, or both strands.

In one embodiment, the invention features a siNA molecule, wherein the sense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or about one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 10 or more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In another embodiment, the invention features a siNA molecule, wherein the sense strand comprises about 1 to about 5, specifically about 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, or more)

universal base modified nucleotides, and optionally a terminal cap molecule at the 3-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5, or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without about 1 to about 5 or more, for example about 1, 2, 3, 4, 5, or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In one embodiment, the invention features a siNA molecule, wherein the antisense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or about one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 10 or more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3' and 5'-ends, being present in the same or different strand.

In another embodiment, the invention features a siNA molecule, wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without about 1 to about 5, for example about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule having about 1 to about 5 or more (specifically about 1, 2, 3, 4, 5 or more) phosphorothioate internucleotide linkages in each strand of the siNA molecule.

In another embodiment, the invention features a siNA molecule comprising 2'-5' internucleotide linkages. The 2'-5' internucleotide linkage(s) can be at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of one or both siNA sequence strands. In addition, the 2'-5' internucleotide linkage(s) can be present at various other positions within one or both siNA sequence strands, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more including every internucleotide linkage of a pyrimidine nucleotide in one or both strands of the siNA molecule can comprise a 2'-5' internucleotide linkage, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more including every internucleotide linkage of a purine nucleotide in one or both strands of the siNA molecule can comprise a 2'-5' internucleotide linkage.

In another embodiment, a chemically-modified siNA molecule of the invention comprises a duplex having two strands, one or both of which can be chemically-modified, wherein each strand is about 18 to about 27 (*e.g.*, about 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27) nucleotides in length, wherein the duplex has about 18 to about 23 (*e.g.*, about 18, 19, 20, 21, 22, or 23) base pairs, and wherein the chemical modification comprises a structure having any of Formulae I-VII. For example, an exemplary chemically-modified siNA molecule of the invention comprises a duplex having two strands, one or both of which can be chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein each strand consists of about 21 nucleotides, each having a 2-nucleotide 3'-terminal nucleotide overhang, and wherein the duplex has about 19 base pairs. In another embodiment, a siNA molecule of the invention comprises a single stranded hairpin structure, wherein the siNA is about 36 to about 70 (*e.g.*, about 36, 40, 45, 50, 55, 60, 65, or 70) nucleotides in length having about 18 to about 23 (*e.g.*, about 18, 19, 20, 21, 22, or 23) base pairs, and wherein the siNA can include a chemical modification comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a linear oligonucleotide having about 42 to about 50 (*e.g.*, about 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides that is chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein the linear oligonucleotide forms a hairpin structure having about 19 base pairs and a 2-nucleotide 3'-terminal nucleotide overhang. In another embodiment, a linear hairpin siNA molecule of the invention contains a stem loop motif, wherein the loop portion of the siNA molecule is biodegradable. For example, a linear hairpin siNA molecule of the invention is designed such that degradation of the loop portion of the siNA molecule *in vivo* can generate a double-stranded siNA molecule with 3'-terminal overhangs, such as 3'-terminal nucleotide overhangs comprising about 2 nucleotides.

In another embodiment, a siNA molecule of the invention comprises a hairpin structure, wherein the siNA is about 25 to about 50 (*e.g.*, about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides in length having about 3 to about 25 (*e.g.*, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) base pairs, and wherein the siNA can include one or

more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a linear oligonucleotide having about 25 to about 35 (*e.g.*, about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35) nucleotides that is chemically-modified with one or more chemical modifications having any of Formulae I-VII or any combination thereof, wherein the linear oligonucleotide forms a hairpin structure having about 3 to about 23 (*e.g.*, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23) base pairs and a 5'-terminal phosphate group that can be chemically modified as described herein (for example a 5'-terminal phosphate group having Formula IV). In another embodiment, a linear hairpin siNA molecule of the invention contains a stem loop motif, wherein the loop portion of the siNA molecule is biodegradable. In one embodiment, a linear hairpin siNA molecule of the invention comprises a loop portion comprising a non-nucleotide linker.

In another embodiment, a siNA molecule of the invention comprises an asymmetric hairpin structure, wherein the siNA is about 25 to about 50 (*e.g.*, about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides in length having about 3 to about 20 (*e.g.*, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) base pairs, and wherein the siNA can include one or more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a linear oligonucleotide having about 25 to about 35 (*e.g.*, about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35) nucleotides that is chemically-modified with one or more chemical modifications having any of Formulae I-VII or any combination thereof, wherein the linear oligonucleotide forms an asymmetric hairpin structure having about 3 to about 18 (*e.g.*, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18) base pairs and a 5'-terminal phosphate group that can be chemically modified as described herein (for example a 5'-terminal phosphate group having Formula IV). In one embodiment, an asymmetric hairpin siNA molecule of the invention contains a stem loop motif, wherein the loop portion of the siNA molecule is biodegradable. In another embodiment, an asymmetric hairpin siNA molecule of the invention comprises a loop portion comprising a non-nucleotide linker.

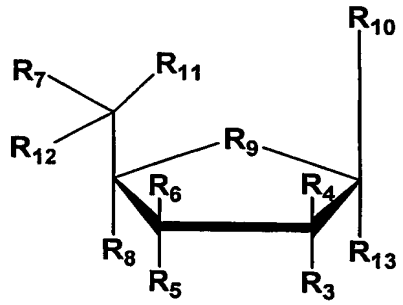
In another embodiment, a siNA molecule of the invention comprises an asymmetric double stranded structure having separate polynucleotide strands comprising sense and antisense regions, wherein the antisense region is about 16 to about 25 (*e.g.*, about 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) nucleotides in length, wherein the sense region is about 3 to about 18 (*e.g.*, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18) nucleotides in length, wherein the sense region and the antisense region have at least 3 complementary nucleotides, and wherein the siNA can include one or more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises an asymmetric double stranded structure having separate polynucleotide strands comprising sense and antisense regions, wherein the antisense region is about 18 to about 22 (*e.g.*, about 18, 19, 20, 21, or 22) nucleotides in length and wherein the sense region is about 3 to about 15 (*e.g.*, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) nucleotides in length, wherein the sense region the antisense region have at least 3 complementary nucleotides, and wherein the siNA can include one or more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. In another embodiment, the asymmetic double stranded siNA molecule can also have a 5'-terminal phosphate group that can be chemically modified as described herein (for example a 5'-terminal phosphate group having Formula IV).

In another embodiment, a siNA molecule of the invention comprises a circular nucleic acid molecule, wherein the siNA is about 38 to about 70 (*e.g.*, about 38, 40, 45, 50, 55, 60, 65, or 70) nucleotides in length having about 18 to about 23 (*e.g.*, about 18, 19, 20, 21, 22,or 23) base pairs, and wherein the siNA can include a chemical modification, which comprises a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a circular oligonucleotide having about 42 to about 50 (*e.g.*, about 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides that is chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein the circular oligonucleotide forms a dumbbell shaped structure having about 19 base pairs and 2 loops.

In another embodiment, a circular siNA molecule of the invention contains two loop motifs, wherein one or both loop portions of the siNA molecule is biodegradable.

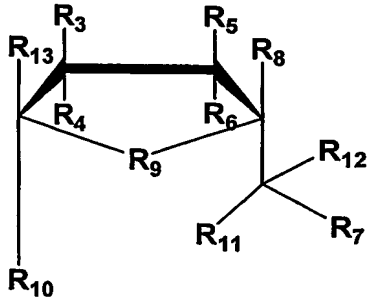
For example, a circular siNA molecule of the invention is designed such that degradation of the loop portions of the siNA molecule *in vivo* can generate a double-stranded siNA molecule with 3'-terminal overhangs, such as 3'-terminal nucleotide overhangs comprising about 2 nucleotides.

In one embodiment, a siNA molecule of the invention comprises at least one (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) abasic moiety, for example a compound having Formula V:



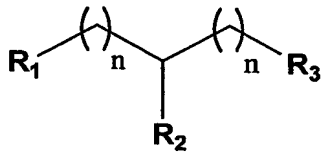
wherein each R3, R4, R5, R6, R7, R8, R10, R11, R12, and R13 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalklylamino, substituted silyl, or group having Formula I or II; R9 is O, S, CH2, S=O, CHF, or CF2.

In one embodiment, a siNA molecule of the invention comprises at least one (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) inverted abasic moiety, for example a compound having Formula VI:



wherein each R3, R4, R5, R6, R7, R8, R10, R11, R12, and R13 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalklylamino, substituted silyl, or group having Formula I or II; R9 is O, S, CH2, S=O, CHF, or CF2, and either R2, R3, R8 or R13 serve as points of attachment to the siNA molecule of the invention.

In another embodiment, a siNA molecule of the invention comprises at least one (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) substituted polyalkyl moieties, for example a compound having Formula VII:



wherein each n is independently an integer from 1 to 12, each R1, R2 and R3 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalklylamino, substituted silyl, or a group having Formula I, and R1, R2 or R3 serves as points of attachment to the siNA molecule of the invention.

In another embodiment, the invention features a compound having Formula VII, wherein R1 and R2 are hydroxyl (OH) groups, n = 1, and R3 comprises O and is the point of attachment to the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both strands of a double-stranded siNA molecule of the invention or to a single-stranded siNA molecule of the invention. This modification is referred to herein as "glyceryl" (for example modification 6 in **Figure 10**).

In another embodiment, a chemically modified nucleoside or non-nucleoside (e.g. a moiety having any of Formula V, VI or VII) of the invention is at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of a siNA molecule of the invention. For example, chemically modified nucleoside or non-nucleoside (e.g., a moiety having Formula V, VI or VII) can be present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense strand, the sense strand, or both antisense and sense strands of the siNA molecule. In one embodiment, the chemically modified nucleoside or non-nucleoside (e.g., a moiety having Formula V, VI or VII) is present at the 5'-end and 3'-end of the sense strand and the 3'-end of the antisense strand of a double stranded siNA molecule of the invention. In one embodiment, the chemically modified nucleoside or non-nucleoside (e.g., a moiety having Formula V, VI or VII) is present at the terminal position of the 5'-end and 3'-end of the sense strand and the 3'-end of the antisense strand of a double stranded siNA molecule of the invention. In one embodiment, the chemically modified nucleoside or non-nucleoside (e.g., a moiety having Formula V, VI or VII) is present at the two terminal positions of the 5'-end and 3'-end of the sense strand and the 3'-end of the antisense strand of a double stranded siNA molecule of the invention. In one embodiment, the chemically modified nucleoside or non-nucleoside (e.g., a moiety having Formula V, VI or VII) is present at the penultimate position of the 5'-end and 3'-end of the sense strand and the 3'-end of the antisense strand of a double stranded siNA molecule of the invention. In addition, a moiety having Formula VII can be present at the 3'-end or the 5'-end of a hairpin siNA molecule as described herein.

In another embodiment, a siNA molecule of the invention comprises an abasic residue having Formula V or VI, wherein the abasic residue having Formula VI or VI is connected to the siNA construct in a 3'-3', 3'-2', 2'-3', or 5'-5' configuration, such as at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both siNA strands.

In one embodiment, a siNA molecule of the invention comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) locked nucleic acid (LNA) nucleotides, for example, at the 5'-end, the 3'-end, both of the 5' and 3'-ends, or any combination thereof, of the siNA molecule.

In another embodiment, a siNA molecule of the invention comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) acyclic nucleotides, for example, at the

5'-end, the 3'-end, both of the 5' and 3'-ends, or any combination thereof, of the siNA molecule.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides), wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said sense region are 2'-deoxy nucleotides.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the sense region are 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), wherein any (*e.g.*, one or more or all) purine nucleotides present in the sense region are 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said sense region are 2'-deoxy nucleotides.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), wherein any (*e.g.*, one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said antisense region are 2'-deoxy nucleotides.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the antisense region are 2'-deoxy purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention capable of mediating RNA interference (RNAi) against BACE inside a cell or reconstituted *in vitro* system comprising a sense region, wherein one or more pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and one or more purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides), and an antisense region, wherein one or more pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and one or more purine nucleotides present in the

antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides). The sense region and/or the antisense region can have a terminal cap modification, such as any modification described herein or shown in **Figure 10**, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense and/or antisense sequence. The sense and/or antisense region can optionally further comprise a 3'-terminal nucleotide overhang having about 1 to about 4 (e.g., about 1, 2, 3, or 4) 2'-deoxynucleotides. The overhang nucleotides can further comprise one or more (e.g., about 1, 2, 3, 4 or more) phosphorothioate, phosphonoacetate, and/or thiophosphonoacetate internucleotide linkages. Non-limiting examples of these chemically-modified siNAs are shown in **Figures 4 and 5** and **Tables III and IV** herein. In any of these described embodiments, the purine nucleotides present in the sense region are alternatively 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides) and one or more purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides). Also, in any of these embodiments, one or more purine nucleotides present in the sense region are alternatively purine ribonucleotides (e.g., wherein all purine nucleotides are purine ribonucleotides or alternately a plurality of purine nucleotides are purine ribonucleotides) and any purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides). Additionally, in any of these embodiments, one or more purine nucleotides present in the sense region and/or present in the antisense region are alternatively selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides (e.g., wherein all purine nucleotides are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides or alternately a plurality of purine nucleotides are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides).

In another embodiment, any modified nucleotides present in the siNA molecules of the invention, preferably in the antisense strand of the siNA molecules of the invention, but also optionally in the sense and/or both antisense and sense strands, comprise modified nucleotides having properties or characteristics similar to naturally occurring ribonucleotides. For example, the invention features siNA molecules including modified nucleotides having a Northern conformation (e.g., Northern pseudorotation cycle, see for example Saenger, *Principles of Nucleic Acid Structure*, Springer-Verlag ed., 1984). As such, chemically modified nucleotides present in the siNA molecules of the invention, preferably in the antisense strand of the siNA molecules of the invention, but also optionally in the sense and/or both antisense and sense strands, are resistant to nuclease degradation while at the same time maintaining the capacity to mediate RNAi. Non-limiting examples of nucleotides having a northern configuration include locked nucleic acid (LNA) nucleotides (e.g., 2′-O, 4′-C-methylene-(D-ribofuranosyl) nucleotides); 2'-methoxyethoxy (MOE) nucleotides; 2'-methyl-thio-ethyl, 2'-deoxy-2'-fluoro nucleotides, 2'-deoxy-2'-chloro nucleotides, 2'-azido nucleotides, and 2'-O-methyl nucleotides.

In one embodiment, the sense strand of a double stranded siNA molecule of the invention comprises a terminal cap moiety, (see for example **Figure 10**) such as an inverted deoxyabaisc moiety, at the 3'-end, 5'-end, or both 3' and 5'-ends of the sense strand.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid molecule (siNA) capable of mediating RNA interference (RNAi) against BACE inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises a conjugate covalently attached to the chemically-modified siNA molecule. Non-limiting examples of conjugates contemplated by the invention include conjugates and ligands described in Vargeese *et al.*, USSN 10/427,160, filed April 30, 2003, incorporated by reference herein in its entirety, including the drawings. In another embodiment, the conjugate is covalently attached to the chemically-modified siNA molecule via a biodegradable linker. In one embodiment, the conjugate molecule is attached at the 3'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule. In another embodiment, the conjugate molecule is attached at the 5'-end of either the sense strand, the antisense strand, or both

strands of the chemically-modified siNA molecule. In yet another embodiment, the conjugate molecule is attached both the 3'-end and 5'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule, or any combination thereof. In one embodiment, a conjugate molecule of the invention comprises a molecule that facilitates delivery of a chemically-modified siNA molecule into a biological system, such as a cell. In another embodiment, the conjugate molecule attached to the chemically-modified siNA molecule is a polyethylene glycol, human serum albumin, or a ligand for a cellular receptor that can mediate cellular uptake. Examples of specific conjugate molecules contemplated by the instant invention that can be attached to chemically-modified siNA molecules are described in Vargeese *et al.*, U.S. Serial No. 10/201,394, filed July 22, 2002 incorporated by reference herein. The type of conjugates used and the extent of conjugation of siNA molecules of the invention can be evaluated for improved pharmacokinetic profiles, bioavailability, and/or stability of siNA constructs while at the same time maintaining the ability of the siNA to mediate RNAi activity. As such, one skilled in the art can screen siNA constructs that are modified with various conjugates to determine whether the siNA conjugate complex possesses improved properties while maintaining the ability to mediate RNAi, for example in animal models as are generally known in the art.

In one embodiment, the invention features a short interfering nucleic acid (siNA) molecule of the invention, wherein the siNA further comprises a nucleotide, non-nucleotide, or mixed nucleotide/non-nucleotide linker that joins the sense region of the siNA to the antisense region of the siNA. In one embodiment, a nucleotide linker of the invention can be a linker of ≥ 2 nucleotides in length, for example about 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in length. In another embodiment, the nucleotide linker can be a nucleic acid aptamer. By "aptamer" or "nucleic acid aptamer" as used herein is meant a nucleic acid molecule that binds specifically to a target molecule wherein the nucleic acid molecule has sequence that comprises a sequence recognized by the target molecule in its natural setting. Alternately, an aptamer can be a nucleic acid molecule that binds to a target molecule where the target molecule does not naturally bind to a nucleic acid. The target molecule can be any molecule of interest. For example, the aptamer can be used to bind to a ligand-binding domain of a protein, thereby preventing interaction of the naturally occurring ligand with the protein. This is a non-limiting example and those

in the art will recognize that other embodiments can be readily generated using techniques generally known in the art. (See, for example, Gold *et al.*, 1995, *Annu. Rev. Biochem.*, 64, 763; Brody and Gold, 2000, *J. Biotechnol.*, 74, 5; Sun, 2000, *Curr. Opin. Mol. Ther.*, 2, 100; Kusser, 2000, *J. Biotechnol.*, 74, 27; Hermann and Patel, 2000, *Science*, 287, 820; and Jayasena, 1999, *Clinical Chemistry*, 45, 1628.)

In yet another embodiment, a non-nucleotide linker of the invention comprises abasic nucleotide, polyether, polyamine, polyamide, peptide, carbohydrate, lipid, polyhydrocarbon, or other polymeric compounds (e.g. polyethylene glycols such as those having between 2 and 100 ethylene glycol units). Specific examples include those described by Seela and Kaiser, *Nucleic Acids Res.* 1990, *18*:6353 and *Nucleic Acids Res.* 1987, *15*:3113; Cload and Schepartz, *J. Am. Chem. Soc.* 1991, *113*:6324; Richardson and Schepartz, *J. Am. Chem. Soc.* 1991, *113*:5109; Ma *et al.*, *Nucleic Acids Res.* 1993, *21*:2585 and *Biochemistry* 1993, *32*:1751; Durand *et al.*, *Nucleic Acids Res.* 1990, *18*:6353; McCurdy *et al.*, *Nucleosides & Nucleotides* 1991, *10*:287; Jschke *et al.*, *Tetrahedron Lett.* 1993, *34*:301; Ono *et al.*, *Biochemistry* 1991, *30*:9914; Arnold *et al.*, International Publication No. WO 89/02439; Usman *et al.*, International Publication No. WO 95/06731; Dudycz *et al.*, International Publication No. WO 95/11910 and Ferentz and Verdine, *J. Am. Chem. Soc.* 1991, *113*:4000, all hereby incorporated by reference herein. A "non-nucleotide" further means any group or compound that can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound can be abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine, for example at the C1 position of the sugar.

In one embodiment, the invention features a short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) inside a cell or reconstituted *in vitro* system, wherein one or both strands of the siNA molecule that are assembled from two separate oligonucleotides do not comprise any ribonucleotides. For example, a siNA molecule can be assembled from a single oligonculeotide where the sense and antisense regions of the siNA comprise separate oligonucleotides that do not have any ribonucleotides (e.g., nucleotides having a 2'-OH group) present in the oligonucleotides. In another example, a siNA molecule can be assembled from a single oligonculeotide

where the sense and antisense regions of the siNA are linked or circularized by a nucleotide or non-nucleotide linker as described herein, wherein the oligonucleotide does not have any ribonucleotides (e.g., nucleotides having a 2'-OH group) present in the oligonucleotide. Applicant has surprisingly found that the presense of ribonucleotides (e.g., nucleotides having a 2'-hydroxyl group) within the siNA molecule is not required or essential to support RNAi activity. As such, in one embodiment, all positions within the siNA can include chemically modified nucleotides and/or non-nucleotides such as nucleotides and or non-nucleotides having Formula I, II, III, IV, V, VI, or VII or any combination thereof to the extent that the ability of the siNA molecule to support RNAi activity in a cell is maintained.

In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted *in vitro* system comprising a single stranded polynucleotide having complementarity to a target nucleic acid sequence. In another embodiment, the single stranded siNA molecule of the invention comprises a 5'-terminal phosphate group. In another embodiment, the single stranded siNA molecule of the invention comprises a 5'-terminal phosphate group and a 3'-terminal phosphate group (e.g., a 2',3'-cyclic phosphate). In another embodiment, the single stranded siNA molecule of the invention comprises about 18 to about 29 (e.g., about 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) nucleotides. In yet another embodiment, the single stranded siNA molecule of the invention comprises one or more chemically modified nucleotides or non-nucleotides described herein. For example, all the positions within the siNA molecule can include chemically-modified nucleotides such as nucleotides having any of Formulae I-VII, or any combination thereof to the extent that the ability of the siNA molecule to support RNAi activity in a cell is maintained.

In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted *in vitro* system comprising a single stranded polynucleotide having complementarity to a target nucleic acid sequence, wherein one or more pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine

nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and a terminal cap modification, such as any modification described herein or shown in **Figure 10**, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence. The siNA optionally further comprises about 1 to about 4 or more (e.g., about 1, 2, 3, 4 or more) terminal 2'-deoxynucleotides at the 3'-end of the siNA molecule, wherein the terminal nucleotides can further comprise one or more (e.g., 1, 2, 3, 4 or more) phosphorothioate, phosphonoacetate, and/or thiophosphonoacetate internucleotide linkages, and wherein the siNA optionally further comprises a terminal phosphate group, such as a 5'-terminal phosphate group. In any of these embodiments, any purine nucleotides present in the antisense region are alternatively 2'-deoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides). Also, in any of these embodiments, any purine nucleotides present in the siNA (i.e., purine nucleotides present in the sense and/or antisense region) can alternatively be locked nucleic acid (LNA) nucleotides (e.g., wherein all purine nucleotides are LNA nucleotides or alternately a plurality of purine nucleotides are LNA nucleotides). Also, in any of these embodiments, any purine nucleotides present in the siNA are alternatively 2'-methoxyethyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-methoxyethyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-methoxyethyl purine nucleotides). In another embodiment, any modified nucleotides present in the single stranded siNA molecules of the invention comprise modified nucleotides having properties or characteristics similar to naturally occurring ribonucleotides. For example, the invention features siNA molecules including modified nucleotides having a Northern conformation (e.g., Northern pseudorotation cycle, see for example Saenger, *Principles of Nucleic Acid Structure*, Springer-Verlag ed., 1984). As such, chemically modified nucleotides present in the single stranded siNA molecules of the invention are preferably resistant to nuclease degradation while at the same time maintaining the capacity to mediate RNAi.

In one embodiment, the invention features a method for modulating the expression of a BACE gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the BACE gene; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the BACE gene in the cell.

In one embodiment, the invention features a method for modulating the expression of a BACE gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the BACE gene and wherein the sense strand sequence of the siNA comprises a sequence identical or substantially similar to the sequence of the target RNA; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the BACE gene in the cell.

In another embodiment, the invention features a method for modulating the expression of more than one BACE gene within a cell comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the BACE genes; and (b) introducing the siNA molecules into a cell under conditions suitable to modulate the expression of the BACE genes in the cell.

In another embodiment, the invention features a method for modulating the expression of two or more BACE genes within a cell comprising: (a) synthesizing one or more siNA molecules of the invention, which can be chemically-modified, wherein the siNA strands comprise sequences complementary to RNA of the BACE genes and wherein the sense strand sequences of the siNAs comprise sequences identical or substantially similar to the sequences of the target RNAs; and (b) introducing the siNA molecules into a cell under conditions suitable to modulate the expression of the BACE genes in the cell.

In another embodiment, the invention features a method for modulating the expression of more than one BACE gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the

siNA strands comprises a sequence complementary to RNA of the BACE gene and wherein the sense strand sequence of the siNA comprises a sequence identical or substantially similar to the sequences of the target RNAs; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the BACE genes in the cell.

In one embodiment, siNA molecules of the invention are used as reagents in *ex vivo* applications. For example, siNA reagents are introduced into tissue or cells that are transplanted into a subject for therapeutic effect. The cells and/or tissue can be derived from an organism or subject that later receives the explant, or can be derived from another organism or subject prior to transplantation. The siNA molecules can be used to modulate the expression of one or more genes in the cells or tissue, such that the cells or tissue obtain a desired phenotype or are able to perform a function when transplanted in vivo. In one embodiment, certain target cells from a patient are extracted. These extracted cells are contacted with siNAs targeting a specific nucleotide sequence within the cells under conditions suitable for uptake of the siNAs by these cells (e.g. using delivery reagents such as cationic lipids, liposomes and the like or using techniques such as electroporation to facilitate the delivery of siNAs into cells). The cells are then reintroduced back into the same patient or other patients. In one embodiment, the invention features a method of modulating the expression of a BACE gene in a tissue explant comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the BACE gene; and (b) introducing the siNA molecule into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the BACE gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the BACE gene in that organism.

In one embodiment, the invention features a method of modulating the expression of a BACE gene in a tissue explant comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the BACE gene and wherein the sense strand sequence of the siNA comprises a sequence identical or substantially similar to the

sequence of the target RNA; and (b) introducing the siNA molecule into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the BACE gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the BACE gene in that organism.

In another embodiment, the invention features a method of modulating the expression of more than one BACE gene in a tissue explant comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the BACE genes; and (b) introducing the siNA molecules into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the BACE genes in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the BACE genes in that organism.

In one embodiment, the invention features a method of modulating the expression of a BACE gene in a subject or organism comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the BACE gene; and (b) introducing the siNA molecule into the subject or organism under conditions suitable to modulate the expression of the BACE gene in the subject or organism. The level of BACE protein or RNA can be determined using various methods well-known in the art.

In another embodiment, the invention features a method of modulating the expression of more than one BACE gene in a subject or organism comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the BACE genes; and (b) introducing the siNA molecules into the subject or organism under conditions suitable to modulate the expression of the BACE genes in the subject or organism. The level of BACE protein or RNA can be determined as is known in the art.

In one embodiment, the invention features a method for modulating the expression of a BACE gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the BACE gene; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the BACE gene in the cell.

In another embodiment, the invention features a method for modulating the expression of more than one BACE gene within a cell comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the BACE gene; and (b) contacting the cell *in vitro* or *in vivo* with the siNA molecule under conditions suitable to modulate the expression of the BACE genes in the cell.

In one embodiment, the invention features a method of modulating the expression of a BACE gene in a tissue explant comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the BACE gene; and (b) contacting a cell of the tissue explant derived from a particular subject or organism with the siNA molecule under conditions suitable to modulate the expression of the BACE gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the subject or organism the tissue was derived from or into another subject or organism under conditions suitable to modulate the expression of the BACE gene in that subject or organism.

In another embodiment, the invention features a method of modulating the expression of more than one BACE gene in a tissue explant comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the BACE gene; and (b) introducing the siNA molecules into a cell of the tissue explant derived from a particular subject or organism under conditions suitable to modulate the expression of the BACE genes in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the subject or organism the

tissue was derived from or into another subject or organism under conditions suitable to modulate the expression of the BACE genes in that subject or organism.

In one embodiment, the invention features a method of modulating the expression of a BACE gene in a subject or organism comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the BACE gene; and (b) introducing the siNA molecule into the subject or organism under conditions suitable to modulate the expression of the BACE gene in the subject or organism.

In another embodiment, the invention features a method of modulating the expression of more than one BACE gene in a subject or organism comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the BACE gene; and (b) introducing the siNA molecules into the subject or organism under conditions suitable to modulate the expression of the BACE genes in the subject or organism.

In one embodiment, the invention features a method of modulating the expression of a BACE gene in a subject or organism comprising contacting the subject or organism with a siNA molecule of the invention under conditions suitable to modulate the expression of the BACE gene in the subject or organism.

In one embodiment, the invention features a method for treating Alzheimer's disease in a subject or organism comprising contacting the subject or organism with a siNA molecule of the invention under conditions suitable to modulate the expression of the BACE gene in the subject or organism.

In one embodiment, the invention features a method for treating neurodegenerative disorders or conditions, such as dementia, in a subject or organism comprising contacting the subject or organism with a siNA molecule of the invention under conditions suitable to modulate the expression of the BACE gene in the subject or organism.

In one embodiment, the invention features a method for treating stroke/cardiovascular accident in a subject or organism comprising contacting the subject

or organism with a siNA molecule of the invention under conditions suitable to modulate the expression of the BACE gene in the subject or organism.

In another embodiment, the invention features a method of modulating the expression of more than one BACE gene in a subject or organism comprising contacting the subject or organism with one or more siNA molecules of the invention under conditions suitable to modulate the expression of the BACE genes in the subject or organism.

The siNA molecules of the invention can be designed to down regulate or inhibit target (e.g., BACE) gene expression through RNAi targeting of a variety of RNA molecules. In one embodiment, the siNA molecules of the invention are used to target various RNAs corresponding to a target gene. Non-limiting examples of such RNAs include messenger RNA (mRNA), alternate RNA splice variants of target gene(s), post-transcriptionally modified RNA of target gene(s), pre-mRNA of target gene(s), and/or RNA templates. If alternate splicing produces a family of transcripts that are distinguished by usage of appropriate exons, the instant invention can be used to inhibit gene expression through the appropriate exons to specifically inhibit or to distinguish among the functions of gene family members. For example, a protein that contains an alternatively spliced transmembrane domain can be expressed in both membrane bound and secreted forms. Use of the invention to target the exon containing the transmembrane domain can be used to determine the functional consequences of pharmaceutical targeting of membrane bound as opposed to the secreted form of the protein. Non-limiting examples of applications of the invention relating to targeting these RNA molecules include therapeutic pharmaceutical applications, pharmaceutical discovery applications, molecular diagnostic and gene function applications, and gene mapping, for example using single nucleotide polymorphism mapping with siNA molecules of the invention. Such applications can be implemented using known gene sequences or from partial sequences available from an expressed sequence tag (EST).

In another embodiment, the siNA molecules of the invention are used to target conserved sequences corresponding to a gene family or gene families such as BACE family genes. As such, siNA molecules targeting multiple BACE targets can provide increased therapeutic effect. In addition, siNA can be used to characterize pathways of

gene function in a variety of applications. For example, the present invention can be used to inhibit the activity of target gene(s) in a pathway to determine the function of uncharacterized gene(s) in gene function analysis, mRNA function analysis, or translational analysis. The invention can be used to determine potential target gene pathways involved in various diseases and conditions toward pharmaceutical development. The invention can be used to understand pathways of gene expression involved in, for example, Alzheimer's disease and other neurodegenerative disorders or conditions, such as dementia, and stroke/cardiovascular accident.

In one embodiment, siNA molecule(s) and/or methods of the invention are used to down regulate the expression of gene(s) that encode RNA referred to by Genbank Accession, for example, BACE genes encoding RNA sequence(s) referred to herein by Genbank Accession number, for example, Genbank Accession Nos. shown in **Table I.**

In one embodiment, the invention features a method comprising: (a) generating a library of siNA constructs having a predetermined complexity; and (b) assaying the siNA constructs of (a) above, under conditions suitable to determine RNAi target sites within the target RNA sequence. In one embodiment, the siNA molecules of (a) have strands of a fixed length, for example, about 23 nucleotides in length. In another embodiment, the siNA molecules of (a) are of differing length, for example having strands of about 18 to about 25 (e.g., about 18, 19, 20, 21, 22, 23, 24, or 25) nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. In another embodiment, fragments of target RNA are analyzed for detectable levels of cleavage, for example by gel electrophoresis, northern blot analysis, or RNAse protection assays, to determine the most suitable target site(s) within the target RNA sequence. The target RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for *in vitro* systems, and by cellular expression in *in vivo* systems.

In one embodiment, the invention features a method comprising: (a) generating a randomized library of siNA constructs having a predetermined complexity, such as of 4^N, where N represents the number of base paired nucleotides in each of the siNA construct strands (*eg.* for a siNA construct having 21 nucleotide sense and antisense strands with

19 base pairs, the complexity would be 4^{19}); and (b) assaying the siNA constructs of (a) above, under conditions suitable to determine RNAi target sites within the target BACE RNA sequence. In another embodiment, the siNA molecules of (a) have strands of a fixed length, for example about 23 nucleotides in length. In yet another embodiment, the siNA molecules of (a) are of differing length, for example having strands of about 18 to about 25 (e.g., about 18, 19, 20, 21, 22, 23, 24, or 25) nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described in Example 7 herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. In another embodiment, fragments of BACE RNA are analyzed for detectable levels of cleavage, for example, by gel electrophoresis, northern blot analysis, or RNAse protection assays, to determine the most suitable target site(s) within the target BACE RNA sequence. The target BACE RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for *in vitro* systems, and by cellular expression in *in vivo* systems.

In another embodiment, the invention features a method comprising: (a) analyzing the sequence of a RNA target encoded by a target gene; (b) synthesizing one or more sets of siNA molecules having sequence complementary to one or more regions of the RNA of (a); and (c) assaying the siNA molecules of (b) under conditions suitable to determine RNAi targets within the target RNA sequence. In one embodiment, the siNA molecules of (b) have strands of a fixed length, for example about 23 nucleotides in length. In another embodiment, the siNA molecules of (b) are of differing length, for example having strands of about 18 to about 25 (e.g., about 18, 19, 20, 21, 22, 23, 24, or 25) nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. Fragments of target RNA are analyzed for detectable levels of cleavage, for example by gel electrophoresis, northern blot analysis, or RNAse protection assays, to determine the most suitable target site(s) within the target RNA sequence. The target RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for *in vitro* systems, and by expression in *in vivo* systems.

By "target site" is meant a sequence within a target RNA that is "targeted" for cleavage mediated by a siNA construct which contains sequences within its antisense region that are complementary to the target sequence.

By "detectable level of cleavage" is meant cleavage of target RNA (and formation of cleaved product RNAs) to an extent sufficient to discern cleavage products above the background of RNAs produced by random degradation of the target RNA. Production of cleavage products from 1-5% of the target RNA is sufficient to detect above the background for most methods of detection.

In one embodiment, the invention features a composition comprising a siNA molecule of the invention, which can be chemically-modified, in a pharmaceutically acceptable carrier or diluent. In another embodiment, the invention features a pharmaceutical composition comprising siNA molecules of the invention, which can be chemically-modified, targeting one or more genes in a pharmaceutically acceptable carrier or diluent. In another embodiment, the invention features a method for diagnosing a disease or condition in a subject comprising administering to the subject a composition of the invention under conditions suitable for the diagnosis of the disease or condition in the subject. In another embodiment, the invention features a method for treating or preventing a disease or condition in a subject, comprising administering to the subject a composition of the invention under conditions suitable for the treatment or prevention of the disease or condition in the subject, alone or in conjunction with one or more other therapeutic compounds. In yet another embodiment, the invention features a method for treating Alzheimer's disease and/or other neurodegenerative disorders, such as dementia and stroke/cardiovascular accident in a subject comprising administering to the subject a composition of the invention under conditions suitable for the treatment of Alzheimer's disease and/or other neurodegenerative disorders, such as dementia and stroke/cardiovascular accident in the subject.

In another embodiment, the invention features a method for validating a BACE gene target, comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands includes a sequence complementary to RNA of a BACE target gene; (b) introducing the siNA molecule into a cell, tissue, subject or organism under conditions suitable for modulating expression of

the BACE target gene in the cell, tissue, subject, or organism; and (c) determining the function of the gene by assaying for any phenotypic change in the cell, tissue, subject, or organism.

In another embodiment, the invention features a method for validating a BACE target comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands includes a sequence complementary to RNA of a BACE target gene; (b) introducing the siNA molecule into a biological system under conditions suitable for modulating expression of the BACE target gene in the biological system; and (c) determining the function of the gene by assaying for any phenotypic change in the biological system.

By "biological system" is meant, material, in a purified or unpurified form, from biological sources, including but not limited to human or animal, wherein the system comprises the components required for RNAi activity. The term "biological system" includes, for example, a cell, tissue, subject, or organism, or extract thereof. The term biological system also includes reconstituted RNAi systems that can be used in an *in vitro* setting.

By "phenotypic change" is meant any detectable change to a cell that occurs in response to contact or treatment with a nucleic acid molecule of the invention (e.g., siNA). Such detectable changes include, but are not limited to, changes in shape, size, proliferation, motility, protein expression or RNA expression or other physical or chemical changes as can be assayed by methods known in the art. The detectable change can also include expression of reporter genes/molecules such as Green Florescent Protein (GFP) or various tags that are used to identify an expressed protein or any other cellular component that can be assayed.

In one embodiment, the invention features a kit containing a siNA molecule of the invention, which can be chemically-modified, that can be used to modulate the expression of a BACE target gene in a biological system, including, for example, in a cell, tissue, subject, or organism. In another embodiment, the invention features a kit containing more than one siNA molecule of the invention, which can be chemically-

modified, that can be used to modulate the expression of more than one BACE target gene in a biological system, including, for example, in a cell, tissue, subject, or organism.

In one embodiment, the invention features a cell containing one or more siNA molecules of the invention, which can be chemically-modified. In another embodiment, the cell containing a siNA molecule of the invention is a mammalian cell. In yet another embodiment, the cell containing a siNA molecule of the invention is a human cell.

In one embodiment, the synthesis of a siNA molecule of the invention, which can be chemically-modified, comprises: (a) synthesis of two complementary strands of the siNA molecule; (b) annealing the two complementary strands together under conditions suitable to obtain a double-stranded siNA molecule. In another embodiment, synthesis of the two complementary strands of the siNA molecule is by solid phase oligonucleotide synthesis. In yet another embodiment, synthesis of the two complementary strands of the siNA molecule is by solid phase tandem oligonucleotide synthesis.

In one embodiment, the invention features a method for synthesizing a siNA duplex molecule comprising: (a) synthesizing a first oligonucleotide sequence strand of the siNA molecule, wherein the first oligonucleotide sequence strand comprises a cleavable linker molecule that can be used as a scaffold for the synthesis of the second oligonucleotide sequence strand of the siNA; (b) synthesizing the second oligonucleotide sequence strand of siNA on the scaffold of the first oligonucleotide sequence strand, wherein the second oligonucleotide sequence strand further comprises a chemical moiety than can be used to purify the siNA duplex; (c) cleaving the linker molecule of (a) under conditions suitable for the two siNA oligonucleotide strands to hybridize and form a stable duplex; and (d) purifying the siNA duplex utilizing the chemical moiety of the second oligonucleotide sequence strand. In one embodiment, cleavage of the linker molecule in (c) above takes place during deprotection of the oligonucleotide, for example, under hydrolysis conditions using an alkylamine base such as methylamine. In one embodiment, the method of synthesis comprises solid phase synthesis on a solid support such as controlled pore glass (CPG) or polystyrene, wherein the first sequence of (a) is synthesized on a cleavable linker, such as a succinyl linker, using the solid support as a scaffold. The cleavable linker in (a) used as a scaffold for synthesizing the second strand can comprise similar reactivity as the solid support derivatized linker, such that

cleavage of the solid support derivatized linker and the cleavable linker of (a) takes place concomitantly. In another embodiment, the chemical moiety of (b) that can be used to isolate the attached oligonucleotide sequence comprises a trityl group, for example a dimethoxytrityl group, which can be employed in a trityl-on synthesis strategy as described herein. In yet another embodiment, the chemical moiety, such as a dimethoxytrityl group, is removed during purification, for example, using acidic conditions.

In a further embodiment, the method for siNA synthesis is a solution phase synthesis or hybrid phase synthesis wherein both strands of the siNA duplex are synthesized in tandem using a cleavable linker attached to the first sequence which acts a scaffold for synthesis of the second sequence. Cleavage of the linker under conditions suitable for hybridization of the separate siNA sequence strands results in formation of the double-stranded siNA molecule.

In another embodiment, the invention features a method for synthesizing a siNA duplex molecule comprising: (a) synthesizing one oligonucleotide sequence strand of the siNA molecule, wherein the sequence comprises a cleavable linker molecule that can be used as a scaffold for the synthesis of another oligonucleotide sequence; (b) synthesizing a second oligonucleotide sequence having complementarity to the first sequence strand on the scaffold of (a), wherein the second sequence comprises the other strand of the double-stranded siNA molecule and wherein the second sequence further comprises a chemical moiety than can be used to isolate the attached oligonucleotide sequence; (c) purifying the product of (b) utilizing the chemical moiety of the second oligonucleotide sequence strand under conditions suitable for isolating the full-length sequence comprising both siNA oligonucleotide strands connected by the cleavable linker and under conditions suitable for the two siNA oligonucleotide strands to hybridize and form a stable duplex. In one embodiment, cleavage of the linker molecule in (c) above takes place during deprotection of the oligonucleotide, for example, under hydrolysis conditions. In another embodiment, cleavage of the linker molecule in (c) above takes place after deprotection of the oligonucleotide. In another embodiment, the method of synthesis comprises solid phase synthesis on a solid support such as controlled pore glass (CPG) or polystyrene, wherein the first sequence of (a) is synthesized on a cleavable linker, such as a succinyl linker, using the solid support as a scaffold. The

cleavable linker in (a) used as a scaffold for synthesizing the second strand can comprise similar reactivity or differing reactivity as the solid support derivatized linker, such that cleavage of the solid support derivatized linker and the cleavable linker of (a) takes place either concomitantly or sequentially. In one embodiment, the chemical moiety of (b) that can be used to isolate the attached oligonucleotide sequence comprises a trityl group, for example a dimethoxytrityl group.

In another embodiment, the invention features a method for making a double-stranded siNA molecule in a single synthetic process comprising: (a) synthesizing an oligonucleotide having a first and a second sequence, wherein the first sequence is complementary to the second sequence, and the first oligonucleotide sequence is linked to the second sequence via a cleavable linker, and wherein a terminal 5'-protecting group, for example, a 5'-O-dimethoxytrityl group (5'-O-DMT) remains on the oligonucleotide having the second sequence; (b) deprotecting the oligonucleotide whereby the deprotection results in the cleavage of the linker joining the two oligonucleotide sequences; and (c) purifying the product of (b) under conditions suitable for isolating the double-stranded siNA molecule, for example using a trityl-on synthesis strategy as described herein.

In another embodiment, the method of synthesis of siNA molecules of the invention comprises the teachings of Scaringe *et al.*, US Patent Nos. 5,889,136; 6,008,400; and 6,111,086, incorporated by reference herein in their entirety.

In one embodiment, the invention features siNA constructs that mediate RNAi against BACE, wherein the siNA construct comprises one or more chemical modifications, for example, one or more chemical modifications having any of Formulae I-VII or any combination thereof that increases the nuclease resistance of the siNA construct.

In another embodiment, the invention features a method for generating siNA molecules with increased nuclease resistance comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased nuclease resistance.

In one embodiment, the invention features siNA constructs that mediate RNAi against BACE, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the sense and antisense strands of the siNA construct.

In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the sense and antisense strands of the siNA molecule comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased binding affinity between the sense and antisense strands of the siNA molecule.

In one embodiment, the invention features siNA constructs that mediate RNAi against BACE, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the antisense strand of the siNA construct and a complementary target RNA sequence within a cell.

In one embodiment, the invention features siNA constructs that mediate RNAi against BACE, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the antisense strand of the siNA construct and a complementary target DNA sequence within a cell.

In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the antisense strand of the siNA molecule and a complementary target RNA sequence comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased binding affinity between the antisense strand of the siNA molecule and a complementary target RNA sequence.

In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the antisense strand of the siNA molecule and a complementary target DNA sequence comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for

isolating siNA molecules having increased binding affinity between the antisense strand of the siNA molecule and a complementary target DNA sequence.

In one embodiment, the invention features siNA constructs that mediate RNAi against BACE, wherein the siNA construct comprises one or more chemical modifications described herein that modulate the polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to the chemically-modified siNA construct.

In another embodiment, the invention features a method for generating siNA molecules capable of mediating increased polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to a chemically-modified siNA molecule comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules capable of mediating increased polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to the chemically-modified siNA molecule.

In one embodiment, the invention features chemically-modified siNA constructs that mediate RNAi against BACE in a cell, wherein the chemical modifications do not significantly effect the interaction of siNA with a target RNA molecule, DNA molecule and/or proteins or other factors that are essential for RNAi in a manner that would decrease the efficacy of RNAi mediated by such siNA constructs.

In another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against BACE comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity.

In yet another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against BACE target RNA comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a

siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity against the target RNA.

In yet another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against BACE target DNA comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity against the target DNA.

In one embodiment, the invention features siNA constructs that mediate RNAi against BACE, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the cellular uptake of the siNA construct.

In another embodiment, the invention features a method for generating siNA molecules against BACE with improved cellular uptake comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved cellular uptake.

In one embodiment, the invention features siNA constructs that mediate RNAi against BACE, wherein the siNA construct comprises one or more chemical modifications described herein that increases the bioavailability of the siNA construct, for example, by attaching polymeric conjugates such as polyethyleneglycol or equivalent conjugates that improve the pharmacokinetics of the siNA construct, or by attaching conjugates that target specific tissue types or cell types *in vivo*. Non-limiting examples of such conjugates are described in Vargeese *et al.*, U.S. Serial No. 10/201,394 incorporated by reference herein.

In one embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability comprising (a) introducing a conjugate into the structure of a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability. Such conjugates can include ligands for cellular receptors, such as peptides derived from naturally occurring protein ligands; protein localization sequences, including cellular ZIP code sequences; antibodies; nucleic acid aptamers; vitamins and

other co-factors, such as folate and N-acetylgalactosamine; polymers, such as polyethyleneglycol (PEG); phospholipids; cholesterol; polyamines, such as spermine or spermidine; and others.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence is chemically modified in a manner that it can no longer act as a guide sequence for efficiently mediating RNA interference and/or be recognized by cellular proteins that facilitate RNAi.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein the second sequence is designed or modified in a manner that prevents its entry into the RNAi pathway as a guide sequence or as a sequence that is complementary to a target nucleic acid (e.g., RNA) sequence. Such design or modifications are expected to enhance the activity of siNA and/or improve the specificity of siNA molecules of the invention. These modifications are also expected to minimize any off-target effects and/or associated toxicity.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence is incapable of acting as a guide sequence for mediating RNA interference.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence does not have a terminal 5'-hydroxyl (5'-OH) or 5'-phosphate group.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence comprises a terminal cap moiety at the 5'-end of said second sequence. In one embodiment, the terminal cap moiety comprises an inverted abasic, inverted deoxy abasic, inverted nucleotide moiety, a group shown in **Figure 10**, an alkyl or cycloalkyl group, a heterocycle, or any other group that prevents RNAi activity in which the second sequence serves as a guide sequence or template for RNAi.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence comprises a terminal cap moiety at the 5'-end and 3'-end of said second sequence. In one embodiment, each terminal cap moiety individually comprises an inverted abasic, inverted deoxy abasic, inverted nucleotide moiety, a group shown in **Figure 10**, an alkyl or cycloalkyl group, a heterocycle, or any other group that prevents RNAi activity in which the second sequence serves as a guide sequence or template for RNAi.

In one embodiment, the invention features a method for generating siNA molecules of the invention with improved specificity for down regulating or inhibiting the expression of a target nucleic acid (e.g., a DNA or RNA such as a gene or its corresponding RNA), comprising (a) introducing one or more chemical modifications into the structure of a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved specificity. In another embodiment, the chemical modification used to improve specificity comprises terminal cap modifications at the 5'-end, 3'-end, or both 5' and 3'-ends of the siNA molecule. The terminal cap modifications can comprise, for example, structures shown in **Figure 10** (e.g. inverted deoxyabasic moieties) or any other chemical modification that renders a portion of the siNA molecule (e.g. the sense strand) incapable of mediating RNA interference against an off target nucleic acid sequence. In a non-limiting example, a siNA molecule is designed such that only the antisense sequence of the siNA molecule can serve as a guide sequence for RISC mediated degradation of a corresponding target

RNA sequence. This can be accomplished by rendering the sense sequence of the siNA inactive by introducing chemical modifications to the sense strand that preclude recognition of the sense strand as a guide sequence by RNAi machinery. In one embodiment, such chemical modifications comprise any chemical group at the 5'-end of the sense strand of the siNA, or any other group that serves to render the sense strand inactive as a guide sequence for mediating RNA interference. These modifications, for example, can result in a molecule where the 5'-end of the sense strand no longer has a free 5'-hydroxyl (5'-OH) or a free 5'-phosphate group (e.g., phosphate, diphosphate, triphosphate, cyclic phosphate etc.). Non-limiting examples of such siNA constructs are described herein, such as "Stab 9/10", "Stab 7/8", "Stab 7/19", "Stab 17/22", "Stab 23/24", and "Stab 24/25" chemistries and variants thereof (see **Table IV**) wherein the 5'-end and 3'-end of the sense strand of the siNA do not comprise a hydroxyl group or phosphate group.

In one embodiment, the invention features a method for generating siNA molecules of the invention with improved specificity for down regulating or inhibiting the expression of a target nucleic acid (e.g., a DNA or RNA such as a gene or its corresponding RNA), comprising introducing one or more chemical modifications into the structure of a siNA molecule that prevent a strand or portion of the siNA molecule from acting as a template or guide sequence for RNAi activity. In one embodiment, the inactive strand or sense region of the siNA molecule is the sense strand or sense region of the siNA molecule, i.e. the strand or region of the siNA that does not have complementarity to the target nucleic acid sequence. In one embodiment, such chemical modifications comprise any chemical group at the 5'-end of the sense strand or region of the siNA that does not comprise a 5'-hydroxyl (5'-OH) or 5'-phosphate group, or any other group that serves to render the sense strand or sense region inactive as a guide sequence for mediating RNA interference. Non-limiting examples of such siNA constructs are described herein, such as "Stab 9/10", "Stab 7/8", "Stab 7/19", "Stab 17/22", "Stab 23/24", and "Stab 24/25" chemistries and variants thereof (see **Table IV**) wherein the 5'-end and 3'-end of the sense strand of the siNA do not comprise a hydroxyl group or phosphate group.

In one embodiment, the invention features a method for screening siNA molecules that are active in mediating RNA interference against a target nucleic acid sequence

comprising (a) generating a plurality of unmodified siNA molecules, (b) screening the siNA molecules of step (a) under conditions suitable for isolating siNA molecules that are active in mediating RNA interference against the target nucleic acid sequence, and (c) introducing chemical modifications (e.g. chemical modifications as described herein or as otherwise known in the art) into the active siNA molecules of (b). In one embodiment, the method further comprises re-screening the chemically modified siNA molecules of step (c) under conditions suitable for isolating chemically modified siNA molecules that are active in mediating RNA interference against the target nucleic acid sequence.

In one embodiment, the invention features a method for screening chemically modified siNA molecules that are active in mediating RNA interference against a target nucleic acid sequence comprising (a) generating a plurality of chemically modified siNA molecules (e.g. siNA molecules as described herein or as otherwise known in the art), and (b) screening the siNA molecules of step (a) under conditions suitable for isolating chemically modified siNA molecules that are active in mediating RNA interference against the target nucleic acid sequence.

The term "ligand" refers to any compound or molecule, such as a drug, peptide, hormone, or neurotransmitter, that is capable of interacting with another compound, such as a receptor, either directly or indirectly. The receptor that interacts with a ligand can be present on the surface of a cell or can alternately be an intercullular receptor. Interaction of the ligand with the receptor can result in a biochemical reaction, or can simply be a physical interaction or association.

In another embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability comprising (a) introducing an excipient formulation to a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability. Such excipients include polymers such as cyclodextrins, lipids, cationic lipids, polyamines, phospholipids, nanoparticles, receptors, ligands, and others.

In another embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability comprising (a) introducing

nucleotides having any of Formulae I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability.

In another embodiment, polyethylene glycol (PEG) can be covalently attached to siNA compounds of the present invention. The attached PEG can be any molecular weight, preferably from about 2,000 to about 50,000 daltons (Da).

The present invention can be used alone or as a component of a kit having at least one of the reagents necessary to carry out the *in vitro* or *in vivo* introduction of RNA to test samples and/or subjects. For example, preferred components of the kit include a siNA molecule of the invention and a vehicle that promotes introduction of the siNA into cells of interest as described herein (e.g., using lipids and other methods of transfection known in the art, see for example Beigelman *et al,* US 6,395,713). The kit can be used for target validation, such as in determining gene function and/or activity, or in drug optimization, and in drug discovery (see for example Usman et al., USSN 60/402,996). Such a kit can also include instructions to allow a user of the kit to practice the invention.

The term "short interfering nucleic acid", "siNA", "short interfering RNA", "siRNA", "short interfering nucleic acid molecule", "short interfering oligonucleotide molecule", or "chemically-modified short interfering nucleic acid molecule" as used herein refers to any nucleic acid molecule capable of inhibiting or down regulating gene expression or viral replication, for example by mediating RNA interference "RNAi" or gene silencing in a sequence-specific manner; see for example Zamore *et al.,* 2000, Cell, 101, 25-33; Bass, 2001, *Nature,* 411, 428-429; Elbashir *et al.,* 2001, *Nature,* 411, 494-498; and Kreutzer *et al.,* International PCT Publication No. WO 00/44895; Zernicka-Goetz *et al.,* International PCT Publication No. WO 01/36646; Fire, International PCT Publication No. WO 99/32619; Plaetinck *et al.,* International PCT Publication No. WO 00/01846; Mello and Fire, International PCT Publication No. WO 01/29058; Deschamps-Depaillette, International PCT Publication No. WO 99/07409; and Li *et al.,* International PCT Publication No. WO 00/44914; Allshire, 2002, *Science,* 297, 1818-1819; Volpe *et al.,* 2002, *Science,* 297, 1833-1837; Jenuwein, 2002, *Science,* 297, 2215-2218; and Hall *et al.,* 2002, *Science,* 297, 2232-2237; Hutvagner and Zamore, 2002, *Science,* 297, 2056-60; McManus *et al.,* 2002, *RNA,* 8, 842-850; Reinhart *et al.,* 2002,

Gene & Dev., 16, 1616-1626; and Reinhart & Bartel, 2002, *Science*, 297, 1831). Non limiting examples of siNA molecules of the invention are shown in **Figures 4-6,** and **Tables II and III** herein. For example the siNA can be a double-stranded polynucleotide molecule comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The siNA can be assembled from two separate oligonucleotides, where one strand is the sense strand and the other is the antisense strand, wherein the antisense and sense strands are self-complementary (i.e. each strand comprises nucleotide sequence that is complementary to nucleotide sequence in the other strand; such as where the antisense strand and sense strand form a duplex or double stranded structure, for example wherein the double stranded region is about 19 base pairs); the antisense strand comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense strand comprises nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. Alternatively, the siNA is assembled from a single oligonucleotide, where the self-complementary sense and antisense regions of the siNA are linked by means of a nucleic acid based or non-nucleic acid-based linker(s). The siNA can be a polynucleotide with a duplex, asymmetric duplex, hairpin or asymmetric hairpin secondary structure, having self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a separate target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The siNA can be a circular single-stranded polynucleotide having two or more loop structures and a stem comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof, and wherein the circular polynucleotide can be processed either *in vivo* or *in vitro* to generate an active siNA molecule capable of mediating RNAi. The siNA can also comprise a single stranded polynucleotide having nucleotide sequence complementary to nucleotide sequence in a target nucleic acid molecule or a portion

thereof (for example, where such siNA molecule does not require the presence within the siNA molecule of nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof), wherein the single stranded polynucleotide can further comprise a terminal phosphate group, such as a 5'-phosphate (see for example Martinez *et al.*, 2002, *Cell.*, 110, 563-574 and Schwarz *et al.*, 2002, *Molecular Cell*, 10, 537-568), or 5',3'-diphosphate. In certain embodiments, the siNA molecule of the invention comprises separate sense and antisense sequences or regions, wherein the sense and antisense regions are covalently linked by nucleotide or non-nucleotide linkers molecules as is known in the art, or are alternately non-covalently linked by ionic interactions, hydrogen bonding, van der waals interactions, hydrophobic interactions, and/or stacking interactions. In certain embodiments, the siNA molecules of the invention comprise nucleotide sequence that is complementary to nucleotide sequence of a target gene. In another embodiment, the siNA molecule of the invention interacts with nucleotide sequence of a target gene in a manner that causes inhibition of expression of the target gene. As used herein, siNA molecules need not be limited to those molecules containing only RNA, but further encompasses chemically-modified nucleotides and non-nucleotides. In certain embodiments, the short interfering nucleic acid molecules of the invention lack 2'-hydroxy (2'-OH) containing nucleotides. Applicant describes in certain embodiments short interfering nucleic acids that do not require the presence of nucleotides having a 2'-hydroxy group for mediating RNAi and as such, short interfering nucleic acid molecules of the invention optionally do not include any ribonucleotides (e.g., nucleotides having a 2'-OH group). Such siNA molecules that do not require the presence of ribonucleotides within the siNA molecule to support RNAi can however have an attached linker or linkers or other attached or associated groups, moieties, or chains containing one or more nucleotides with 2'-OH groups. Optionally, siNA molecules can comprise ribonucleotides at about 5, 10, 20, 30, 40, or 50% of the nucleotide positions. The modified short interfering nucleic acid molecules of the invention can also be referred to as short interfering modified oligonucleotides "siMON." As used herein, the term siNA is meant to be equivalent to other terms used to describe nucleic acid molecules that are capable of mediating sequence specific RNAi, for example short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), short hairpin RNA (shRNA), short interfering oligonucleotide, short interfering nucleic acid, short interfering modified oligonucleotide, chemically-modified

siRNA, post-transcriptional gene silencing RNA (ptgsRNA), and others. In addition, as used herein, the term RNAi is meant to be equivalent to other terms used to describe sequence specific RNA interference, such as post transcriptional gene silencing, translational inhibition, or epigenetics. For example, siNA molecules of the invention can be used to epigenetically silence genes at both the post-transcriptional level or the pre-transcriptional level. In a non-limiting example, epigenetic regulation of gene expression by siNA molecules of the invention can result from siNA mediated modification of chromatin structure or methylation pattern to alter gene expression (see, for example, Verdel *et al.*, 2004, *Science*, 303, 672-676; Pal-Bhadra *et al.*, 2004, *Science*, 303, 669-672; Allshire, 2002, *Science*, 297, 1818-1819; Volpe *et al.*, 2002, *Science*, 297, 1833-1837; Jenuwein, 2002, *Science*, 297, 2215-2218; and Hall *et al.*, 2002, *Science*, 297, 2232-2237).

In one embodiment, a siNA molecule of the invention is a duplex forming oligonucleotide "DFO", (see for example Figures 14-15 and Vaish et al., USSN 10/727,780 filed December 3, 2003 and International PCT Application No. US04/16390, filed May 24, 2004).

In one embodiment, a siNA molecule of the invention is a multifunctional siNA, (see for example Figures 16-21 and Jadhav *et al.*, USSN 60/543,480 filed February 10, 2004 and International PCT Application No. US04/16390, filed May 24, 2004). The multifunctional siNA of the invention can comprise sequence targeting, for example, two regions of BACE RNA (see for example target sequences in **Tables II and III**).

By "asymmetric hairpin" as used herein is meant a linear siNA molecule comprising an antisense region, a loop portion that can comprise nucleotides or non-nucleotides, and a sense region that comprises fewer nucleotides than the antisense region to the extent that the sense region has enough complementary nucleotides to base pair with the antisense region and form a duplex with loop. For example, an asymmetric hairpin siNA molecule of the invention can comprise an antisense region having length sufficient to mediate RNAi in a cell or in vitro system (e.g. about 19 to about 22, or about 19, 20, 21, or 22 nucleotides) and a loop region comprising about 4 to about 8 (e.g., about 4, 5, 6, 7, or 8) nucleotides, and a sense region having about 3 to about 18 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18) nucleotides that are

complementary to the antisense region. The asymmetric hairpin siNA molecule can also comprise a 5'-terminal phosphate group that can be chemically modified. The loop portion of the asymmetric hairpin siNA molecule can comprise nucleotides, non-nucleotides, linker molecules, or conjugate molecules as described herein.

By "asymmetric duplex" as used herein is meant a siNA molecule having two separate strands comprising a sense region and an antisense region, wherein the sense region comprises fewer nucleotides than the antisense region to the extent that the sense region has enough complementary nucleotides to base pair with the antisense region and form a duplex. For example, an asymmetric duplex siNA molecule of the invention can comprise an antisense region having length sufficient to mediate RNAi in a cell or in vitro system e.g. about 19 to about 22 (e.g. about 19, 20, 21, or 22) nucleotides and a sense region having about 3 to about 18 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18) nucleotides that are complementary to the antisense region.

By "modulate" is meant that the expression of the gene, or level of RNA molecule or equivalent RNA molecules encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits is up regulated or down regulated, such that expression, level, or activity is greater than or less than that observed in the absence of the modulator. For example, the term "modulate" can mean "inhibit," but the use of the word "modulate" is not limited to this definition.

By "inhibit", "down-regulate", or "reduce", it is meant that the expression of the gene, or level of RNA molecules or equivalent RNA molecules encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits, is reduced below that observed in the absence of the nucleic acid molecules (e.g., siNA) of the invention. In one embodiment, inhibition, down-regulation or reduction with an siNA molecule is below that level observed in the presence of an inactive or attenuated molecule. In another embodiment, inhibition, down-regulation, or reduction with siNA molecules is below that level observed in the presence of, for example, an siNA molecule with scrambled sequence or with mismatches. In another embodiment, inhibition, down-regulation, or reduction of gene expression with a nucleic acid molecule of the instant invention is greater in the presence of the nucleic acid molecule than in its absence. In one embodiment, inhibition, down regulation, or reduction of gene

expression is associated with post transcriptional silencing, such as RNAi mediated cleavage of a target nucleic acid molecule (e.g. RNA) or inhibition of translation. In one embodiment, inhibition, down regulation, or reduction of gene expression is associated with pretranscriptional silencing.

By "gene", or "target gene", is meant, a nucleic acid that encodes an RNA, for example, nucleic acid sequences including, but not limited to, structural genes encoding a polypeptide. A gene or target gene can also encode a functional RNA (fRNA) or non-coding RNA (ncRNA), such as small temporal RNA (stRNA), micro RNA (miRNA), small nuclear RNA (snRNA), short interfering RNA (siRNA), small nucleolar RNA (snRNA), ribosomal RNA (rRNA), transfer RNA (tRNA) and precursor RNAs thereof. Such non-coding RNAs can serve as target nucleic acid molecules for siNA mediated RNA interference in modulating the activity of fRNA or ncRNA involved in functional or regulatory cellular processes. Abberant fRNA or ncRNA activity leading to disease can therefore be modulated by siNA molecules of the invention. siNA molecules targeting fRNA and ncRNA can also be used to manipulate or alter the genotype or phenotype of a subject, organism or cell, by intervening in cellular processes such as genetic imprinting, transcription, translation, or nucleic acid processing (e.g., transamination, methylation etc.). The target gene can be a gene derived from a cell, an endogenous gene, a transgene, or exogenous genes such as genes of a pathogen, for example a virus, which is present in the cell after infection thereof. The cell containing the target gene can be derived from or contained in any organism, for example a plant, animal, protozoan, virus, bacterium, or fungus. Non-limiting examples of plants include monocots, dicots, or gymnosperms. Non-limiting examples of animals include vertebrates or invertebrates. Non-limiting examples of fungi include molds or yeasts. For a review, see for example Snyder and Gerstein, 2003, *Science,* 300, 258-260.

By "non-canonical base pair" is meant any non-Watson Crick base pair, such as mismatches and/or wobble base pairs, inlcuding flipped mismatches, single hydrogen bond mismatches, trans-type mismatches, triple base interactions, and quadruple base interactions. Non-limiting examples of such non-canonical base pairs include, but are not limited to, AC reverse Hoogsteen, AC wobble, AU reverse Hoogsteen, GU wobble, AA N7 amino, CC 2-carbonyl-amino(H1)-N3-amino(H2), GA sheared, UC 4-carbonyl-amino, UU imino-carbonyl, AC reverse wobble, AU Hoogsteen, AU reverse Watson

Crick, CG reverse Watson Crick, GC N3-amino-amino N3, AA N1-amino symmetric, AA N7-amino symmetric, GA N7-N1 amino-carbonyl, GA+ carbonyl-amino N7-N1, GG N1-carbonyl symmetric, GG N3-amino symmetric, CC carbonyl-amino symmetric, CC N3-amino symmetric, UU 2-carbonyl-imino symmetric, UU 4-carbonyl-imino symmetric, AA amino-N3, AA N1-amino, AC amino 2-carbonyl, AC N3-amino, AC N7-amino, AU amino-4-carbonyl, AU N1-imino, AU N3-imino, AU N7-imino, CC carbonyl-amino, GA amino-N1, GA amino-N7, GA carbonyl-amino, GA N3-amino, GC amino-N3, GC carbonyl-amino, GC N3-amino, GC N7-amino, GG amino-N7, GG carbonyl-imino, GG N7-amino, GU amino-2-carbonyl, GU carbonyl-imino, GU imino-2-carbonyl, GU N7-imino, psiU imino-2-carbonyl, UC 4-carbonyl-amino, UC imino-carbonyl, UU imino-4-carbonyl, AC C2-H-N3, GA carbonyl-C2-H, UU imino-4-carbonyl 2 carbonyl-C5-H, AC amino(A) N3(C)-carbonyl, GC imino amino-carbonyl, Gpsi imino-2-carbonyl amino-2- carbonyl, and GU imino amino-2-carbonyl base pairs.

By "BACE" or "beta secretase"as used herein is meant, BACE protein, peptide, or polypeptide having beta-secretase activity, such as that involved in generating beta-amyloid, for example, sequences encoded by BACE Genbank Accession Nos. shown in **Table I.** The term BACE also refers to nucleic acid sequences encoding any BACE protein, peptide, or polypeptide having BACE activity. The term "BACE" is also meant to include other BACE encoding sequence, such as BACE isoforms, mutant BACE genes, splice variants of BACE genes, and BACE gene polymorphisms.

By "APP" or "amyloid precursor protein" as used herein is meant any protein, peptide, or polypeptide that is processed to generate beta-amyloid. The term APP also refers to sequences that encode APP protein, for example, Genbank Accession Nos. shown in **Table I.** The term APP also refers to nucleic acid sequences encoding any APP protein, peptide, or polypeptide having APP activity. The term "APP" is also meant to include other APP encoding sequence, such as APP isoforms, mutant APP genes, splice variants of APP, and APP gene polymorphisms.

By "presenillin" or "PS", i.e, "PS-1" or "PS-2", or "PSEN", i.e., "PSEN1" or "PSEN2", as used herein is meant any protein, peptide, or polypeptide having gamma-secretase activity, such as that involved in generating beta-amyloid. The term PS also refers to sequences that encode presenillin protein, for example, PS-1 or PS-2, (i.e.,

Genbank Accession Nos. shown in **Table I**). The term "PS", for example, "PS-1" or "PS-2", also refers to nucleic acid sequences encoding any PS protein, peptide, or polypeptide having PS activity. The term "PS", for example, "PS-1" or "PS-2", is also meant to include other PS encoding sequence, such as PS isoforms, mutant PS genes, splice variants of PS, and PS gene polymorphisms.

By "PIN-1" as used herein is meant any protein, peptide, or polypeptide having peptidyl-prolyl cis/trans isomerase activity, such as those involved in the development of Neurofibrillary Tangles. The term PIN-1 also refers to sequences that encode PIN-1 protein, i.e., Genbank Accession Nos. shown in **Table I**. The term PIN-1 also refers to nucleic acid sequences encoding any PIN-1 protein, peptide, or polypeptide having PIN-1 activity. The term "PIN-1" is also meant to include other PIN-1 encoding sequence, such as PIN-1 isoforms, mutant PIN-1 genes, splice variants of PIN-1, and PIN-1 gene polymorphisms.

Furthermore, as discussed previously, all embodiments, compositions, methods, and uses described herein using BACE as an examplery gene are equally applicable to APP, PIN-1, and PS (i.e., PS-1, and PS-2) genes.

By "homologous sequence" is meant, a nucleotide sequence that is shared by one or more polynucleotide sequences, such as genes, gene transcripts and/or non-coding polynucleotides. For example, a homologous sequence can be a nucleotide sequence that is shared by two or more genes encoding related but different proteins, such as different members of a gene family, different protein epitopes, different protein isoforms or completely divergent genes, such as a cytokine and its corresponding receptors. A homologous sequence can be a nucleotide sequence that is shared by two or more non-coding polynucleotides, such as noncoding DNA or RNA, regulatory sequences, introns, and sites of transcriptional control or regulation. Homologous sequences can also include conserved sequence regions shared by more than one polynucleotide sequence. Homology does not need to be perfect homology (e.g., 100%), as partially homologous sequences are also contemplated by the instant invention (e.g., 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80% etc.).

By "conserved sequence region" is meant, a nucleotide sequence of one or more regions in a polynucleotide does not vary significantly between generations or from one biological system, subject, or organism to another biological system, subject, or organism. The polynucleotide can include both coding and non-coding DNA and RNA.

By "sense region" is meant a nucleotide sequence of a siNA molecule having complementarity to an antisense region of the siNA molecule. In addition, the sense region of a siNA molecule can comprise a nucleic acid sequence having homology with a target nucleic acid sequence.

By "antisense region" is meant a nucleotide sequence of a siNA molecule having complementarity to a target nucleic acid sequence. In addition, the antisense region of a siNA molecule can optionally comprise a nucleic acid sequence having complementarity to a sense region of the siNA molecule.

By "target nucleic acid" is meant any nucleic acid sequence whose expression or activity is to be modulated. The target nucleic acid can be DNA or RNA.

By "complementarity" is meant that a nucleic acid can form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick or other non-traditional types. In reference to the nucleic molecules of the present invention, the binding free energy for a nucleic acid molecule with its complementary sequence is sufficient to allow the relevant function of the nucleic acid to proceed, e.g., RNAi activity. Determination of binding free energies for nucleic acid molecules is well known in the art (see, e.g., Turner *et al.*, 1987, *CSH Symp. Quant. Biol.* LII pp.123-133; Frier *et al.*, 1986, *Proc. Nat. Acad. Sci.* USA 83:9373-9377; Turner *et al.*, 1987, *J. Am. Chem. Soc.* 109:3783-3785). A percent complementarity indicates the percentage of contiguous residues in a nucleic acid molecule that can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9, or 10 nucleotides out of a total of 10 nucleotides in the first oligonucleotide being based paired to a second nucleic acid sequence having 10 nucleotides represents 50%, 60%, 70%, 80%, 90%, and 100% complementary respectively). "Perfectly complementary" means that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence.

In one embodiment, siNA molecules of the invention that down regulate or reduce BACE gene expression are used for treating Alzheimer's disease in a subject or organism.

In one embodiment, the siNA molecules of the invention are used to treat neurodegenerative disorders or conditions, such as dementia, and stroke/cardiovascular accident in a subject or organism.

In one embodiment of the present invention, each sequence of a siNA molecule of the invention is independently about 18 to about 24 nucleotides in length, in specific embodiments about 18, 19, 20, 21, 22, 23, or 24 nucleotides in length. In another embodiment, the siNA duplexes of the invention independently comprise about 17 to about 23 base pairs (*e.g.*, about 17, 18, 19, 20, 21, 22, or 23). In yet another embodiment, siNA molecules of the invention comprising hairpin or circular structures are about 35 to about 55 (*e.g.*, about 35, 40, 45, 50 or 55) nucleotides in length, or about 38 to about 44 (*e.g.*, about 38, 39, 40, 41, 42, 43, or 44) nucleotides in length and comprising about 16 to about 22 (*e.g.*, about 16, 17, 18, 19, 20, 21 or 22) base pairs. Exemplary siNA molecules of the invention are shown in **Table II**. Exemplary synthetic siNA molecules of the invention are shown in **Table III** and/or **Figures 4-5**.

As used herein "cell" is used in its usual biological sense, and does not refer to an entire multicellular organism, e.g., specifically does not refer to a human. The cell can be present in an organism, e.g., birds, plants and mammals such as humans, cows, sheep, apes, monkeys, swine, dogs, and cats. The cell can be prokaryotic (e.g., bacterial cell) or eukaryotic (e.g., mammalian or plant cell). The cell can be of somatic or germ line origin, totipotent or pluripotent, dividing or non-dividing. The cell can also be derived from or can comprise a gamete or embryo, a stem cell, or a fully differentiated cell.

The siNA molecules of the invention are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells or tissues. The nucleic acid or nucleic acid complexes can be locally administered to relevant tissues *ex vivo*, or *in vivo* through direct dermal application, transdermal application, or injection, with or without their incorporation in biopolymers. In particular embodiments, the nucleic acid molecules of the invention comprise sequences

shown in **Tables II-III** and/or **Figures 4-5**. Examples of such nucleic acid molecules consist essentially of sequences defined in these tables and figures. Furthermore, the chemically modified constructs described in **Table IV** can be applied to any siNA sequence of the invention.

In another aspect, the invention provides mammalian cells containing one or more siNA molecules of this invention. The one or more siNA molecules can independently be targeted to the same or different sites.

By "RNA" is meant a molecule comprising at least one ribonucleotide residue. By "ribonucleotide" is meant a nucleotide with a hydroxyl group at the 2' position of a β-D-ribofuranose moiety. The terms include double-stranded RNA, single-stranded RNA, isolated RNA such as partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA, as well as altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of the siNA or internally, for example at one or more nucleotides of the RNA. Nucleotides in the RNA molecules of the instant invention can also comprise non-standard nucleotides, such as non-naturally occurring nucleotides or chemically synthesized nucleotides or deoxynucleotides. These altered RNAs can be referred to as analogs or analogs of naturally-occurring RNA.

By "subject" is meant an organism, which is a donor or recipient of explanted cells or the cells themselves. "Subject" also refers to an organism to which the nucleic acid molecules of the invention can be administered. A subject can be a mammal or mammalian cells, including a human or human cells.

The term "phosphorothioate" as used herein refers to an internucleotide linkage having Formula I, wherein Z and/or W comprise a sulfur atom. Hence, the term phosphorothioate refers to both phosphorothioate and phosphorodithioate internucleotide linkages.

The term "phosphonoacetate" as used herein refers to an internucleotide linkage having Formula I, wherein Z and/or W comprise an acetyl or protected acetyl group.

The term "thiophosphonoacetate" as used herein refers to an internucleotide linkage having Formula I, wherein Z comprises an acetyl or protected acetyl group and W comprises a sulfur atom or alternately W comprises an acetyl or protected acetyl group and Z comprises a sulfur atom.

The term "universal base" as used herein refers to nucleotide base analogs that form base pairs with each of the natural DNA/RNA bases with little discrimination between them. Non-limiting examples of universal bases include C-phenyl, C-naphthyl and other aromatic derivatives, inosine, azole carboxamides, and nitroazole derivatives such as 3-nitropyrrole, 4-nitroindole, 5-nitroindole, and 6-nitroindole as known in the art (see for example Loakes, 2001, *Nucleic Acids Research,* 29, 2437-2447).

The term "acyclic nucleotide" as used herein refers to any nucleotide having an acyclic ribose sugar, for example where any of the ribose carbons (C1, C2, C3, C4, or C5), are independently or in combination absent from the nucleotide.

The nucleic acid molecules of the instant invention, individually, or in combination or in conjunction with other drugs, can be used to for preventing or treating Alzheimer's disease and other neurodegenerative disorders or conditions, such as dementia and stroke/cardiovascular accident in a subject or organism.

For example, the siNA molecules can be administered to a subject or can be administered to other appropriate cells evident to those skilled in the art, individually or in combination with one or more drugs under conditions suitable for the treatment.

In a further embodiment, the siNA molecules can be used in combination with other known treatments to prevent or treat Alzheimer's disease and other neurodegenerative disorders or conditions, such as dementia and stroke/cardiovascular accident in a subject or organism. For example, the described molecules could be used in combination with one or more known compounds, treatments, or procedures to prevent or treat Alzheimer's disease and other neurodegenerative disorders or conditions, such as dementia and stroke/cardiovascular accident in a subject or organism as are known in the art.

In one embodiment, the invention features an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the invention, in a manner which allows expression of the siNA molecule. For example, the vector can contain sequence(s) encoding both strands of a siNA molecule comprising a duplex. The vector can also contain sequence(s) encoding a single nucleic acid molecule that is self-complementary and thus forms a siNA molecule. Non-limiting examples of such expression vectors are described in Paul *et al.*, 2002, *Nature Biotechnology,* 19, 505; Miyagishi and Taira, 2002, *Nature Biotechnology,* 19, 497; Lee *et al.*, 2002, *Nature Biotechnology,* 19, 500; and Novina *et al.*, 2002, *Nature Medicine,* advance online publication doi:10.1038/nm725.

In another embodiment, the invention features a mammalian cell, for example, a human cell, including an expression vector of the invention.

In yet another embodiment, the expression vector of the invention comprises a sequence for a siNA molecule having complementarity to a RNA molecule referred to by a Genbank Accession numbers, for example Genbank Accession Nos. shown in **Table I.**

In one embodiment, an expression vector of the invention comprises a nucleic acid sequence encoding two or more siNA molecules, which can be the same or different.

In another aspect of the invention, siNA molecules that interact with target RNA molecules and down-regulate gene encoding target RNA molecules (for example target RNA molecules referred to by Genbank Accession numbers herein) are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. The recombinant vectors capable of expressing the siNA molecules can be delivered as described herein, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of siNA molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the siNA molecules bind and down-regulate gene function or expression via RNA interference (RNAi). Delivery of siNA expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from a subject followed by reintroduction into

the subject, or by any other means that would allow for introduction into the desired target cell.

By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a non-limiting example of a scheme for the synthesis of siNA molecules. The complementary siNA sequence strands, strand 1 and strand 2, are synthesized in tandem and are connected by a cleavable linkage, such as a nucleotide succinate or abasic succinate, which can be the same or different from the cleavable linker used for solid phase synthesis on a solid support. The synthesis can be either solid phase or solution phase, in the example shown, the synthesis is a solid phase synthesis. The synthesis is performed such that a protecting group, such as a dimethoxytrityl group, remains intact on the terminal nucleotide of the tandem oligonucleotide. Upon cleavage and deprotection of the oligonucleotide, the two siNA strands spontaneously hybridize to form a siNA duplex, which allows the purification of the duplex by utilizing the properties of the terminal protecting group, for example by applying a trityl on purification method wherein only duplexes/oligonucleotides with the terminal protecting group are isolated.

Figure 2 shows a MALDI-TOF mass spectrum of a purified siNA duplex synthesized by a method of the invention. The two peaks shown correspond to the predicted mass of the separate siNA sequence strands. This result demonstrates that the siNA duplex generated from tandem synthesis can be purified as a single entity using a simple trityl-on purification methodology.

Figure 3 shows a non-limiting proposed mechanistic representation of target RNA degradation involved in RNAi. Double-stranded RNA (dsRNA), which is generated by RNA-dependent RNA polymerase (RdRP) from foreign single-stranded RNA, for example viral, transposon, or other exogenous RNA, activates the DICER enzyme that in

turn generates siNA duplexes. Alternately, synthetic or expressed siNA can be introduced directly into a cell by appropriate means. An active siNA complex forms which recognizes a target RNA, resulting in degradation of the target RNA by the RISC endonuclease complex or in the synthesis of additional RNA by RNA-dependent RNA polymerase (RdRP), which can activate DICER and result in additional siNA molecules, thereby amplifying the RNAi response.

Figure 4A-F shows non-limiting examples of chemically-modified siNA constructs of the present invention. In the figure, N stands for any nucleotide (adenosine, guanosine, cytosine, uridine, or optionally thymidine, for example thymidine can be substituted in the overhanging regions designated by parenthesis (N N). Various modifications are shown for the sense and antisense strands of the siNA constructs.

Figure 4A: The sense strand comprises 21 nucleotides wherein the two terminal 3'-nucleotides are optionally base paired and wherein all nucleotides present are ribonucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all nucleotides present are ribonucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the antisense strand.

Figure 4B: The sense strand comprises 21 nucleotides wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all

pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the sense and antisense strand.

Figure 4C: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-O-methyl or 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the antisense strand.

Figure 4D: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein and wherein and all purine nucleotides that may be present are 2'-deoxy nucleotides. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise

ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the antisense strand.

Figure 4E: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the antisense strand.

Figure 4F: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein and wherein and all purine nucleotides that may be present are 2'-deoxy nucleotides. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and having one 3'-terminal phosphorothioate internucleotide linkage and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-deoxy nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or

other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the antisense strand. The antisense strand of constructs A-F comprise sequence complementary to any target nucleic acid sequence of the invention. Furthermore, when a glyceryl moiety (L) is present at the 3'-end of the antisense strand for any construct shown in Figure 4 A-F, the modified internucleotide linkage is optional.

Figure 5A-F shows non-limiting examples of specific chemically-modified siNA sequences of the invention. **A-F** applies the chemical modifications described in **Figure 4A-F** to a BACE siNA sequence. Such chemical modifications can be applied to any BACE sequence and/or BACE polymorphism sequence.

Figure 6 shows non-limiting examples of different siNA constructs of the invention. The examples shown (constructs 1, 2, and 3) have 19 representative base pairs; however, different embodiments of the invention include any number of base pairs described herein. Bracketed regions represent nucleotide overhangs, for example, comprising about 1, 2, 3, or 4 nucleotides in length, preferably about 2 nucleotides. Constructs 1 and 2 can be used independently for RNAi activity. Construct 2 can comprise a polynucleotide or non-nucleotide linker, which can optionally be designed as a biodegradable linker. In one embodiment, the loop structure shown in construct 2 can comprise a biodegradable linker that results in the formation of construct 1 *in vivo* and/or *in vitro*. In another example, construct 3 can be used to generate construct 2 under the same principle wherein a linker is used to generate the active siNA construct 2 *in vivo* and/or *in vitro*, which can optionally utilize another biodegradable linker to generate the active siNA construct 1 *in vivo* and/or *in vitro*. As such, the stability and/or activity of the siNA constructs can be modulated based on the design of the siNA construct for use *in vivo* or *in vitro* and/or *in vitro*.

Figure 7A-C is a diagrammatic representation of a scheme utilized in generating an expression cassette to generate siNA hairpin constructs.

Figure 7A: A DNA oligomer is synthesized with a 5'-restriction site (R1) sequence followed by a region having sequence identical (sense region of siNA) to a predetermined BACE target sequence, wherein the sense region comprises, for example,

about 19, 20, 21, or 22 nucleotides (N) in length, which is followed by a loop sequence of defined sequence (X), comprising, for example, about 3 to about 10 nucleotides.

Figure 7B: The synthetic construct is then extended by DNA polymerase to generate a hairpin structure having self-complementary sequence that will result in a siNA transcript having specificity for a BACE target sequence and having self-complementary sense and antisense regions.

Figure 7C: The construct is heated (for example to about 95°C) to linearize the sequence, thus allowing extension of a complementary second DNA strand using a primer to the 3'-restriction sequence of the first strand. The double-stranded DNA is then inserted into an appropriate vector for expression in cells. The construct can be designed such that a 3'-terminal nucleotide overhang results from the transcription, for example, by engineering restriction sites and/or utilizing a poly-U termination region as described in Paul *et al.*, 2002, *Nature Biotechnology*, 29, 505-508.

Figure 8A-C is a diagrammatic representation of a scheme utilized in generating an expression cassette to generate double-stranded siNA constructs.

Figure 8A: A DNA oligomer is synthesized with a 5'-restriction (R1) site sequence followed by a region having sequence identical (sense region of siNA) to a predetermined BACE target sequence, wherein the sense region comprises, for example, about 19, 20, 21, or 22 nucleotides (N) in length, and which is followed by a 3'-restriction site (R2) which is adjacent to a loop sequence of defined sequence (X).

Figure 8B: The synthetic construct is then extended by DNA polymerase to generate a hairpin structure having self-complementary sequence.

Figure 8C: The construct is processed by restriction enzymes specific to R1 and R2 to generate a double-stranded DNA which is then inserted into an appropriate vector for expression in cells. The transcription cassette is designed such that a U6 promoter region flanks each side of the dsDNA which generates the separate sense and antisense strands of the siNA. Poly T termination sequences can be added to the constructs to generate U overhangs in the resulting transcript.

Figure 9A-E is a diagrammatic representation of a method used to determine target sites for siNA mediated RNAi within a particular target nucleic acid sequence, such as messenger RNA.

Figure 9A: A pool of siNA oligonucleotides are synthesized wherein the antisense region of the siNA constructs has complementarity to target sites across the target nucleic acid sequence, and wherein the sense region comprises sequence complementary to the antisense region of the siNA.

Figure 9B&C: **(Figure 9B)** The sequences are pooled and are inserted into vectors such that **(Figure 9C)** transfection of a vector into cells results in the expression of the siNA.

Figure 9D: Cells are sorted based on phenotypic change that is associated with modulation of the target nucleic acid sequence.

Figure 9E: The siNA is isolated from the sorted cells and is sequenced to identify efficacious target sites within the target nucleic acid sequence.

Figure 10 shows non-limiting examples of different stabilization chemistries (1-10) that can be used, for example, to stabilize the 3'-end of siNA sequences of the invention, including **(1)** [3-3']-inverted deoxyribose; **(2)** deoxyribonucleotide; **(3)** [5'-3']-3'-deoxyribonucleotide; **(4)** [5'-3']-ribonucleotide; **(5)** [5'-3']-3'-O-methyl ribonucleotide; **(6)** 3'-glyceryl; **(7)** [3'-5']-3'-deoxyribonucleotide; **(8)** [3'-3']-deoxyribonucleotide; **(9)** [5'-2']-deoxyribonucleotide; and **(10)** [5-3']-dideoxyribonucleotide. In addition to modified and unmodified backbone chemistries indicated in the figure, these chemistries can be combined with different backbone modifications as described herein, for example, backbone modifications having Formula I. In addition, the 2'-deoxy nucleotide shown 5' to the terminal modifications shown can be another modified or unmodified nucleotide or non-nucleotide described herein, for example modifications having any of Formulae I-VII or any combination thereof.

Figure 11 shows a non-limiting example of a strategy used to identify chemically modified siNA constructs of the invention that are nuclease resistance while preserving the ability to mediate RNAi activity. Chemical modifications are introduced into the

siNA construct based on educated design parameters (e.g. introducing 2'-mofications, base modifications, backbone modifications, terminal cap modifications etc). The modified construct in tested in an appropriate system (e.g. human serum for nuclease resistance, shown, or an animal model for PK/delivery parameters). In parallel, the siNA construct is tested for RNAi activity, for example in a cell culture system such as a luciferase reporter assay). Lead siNA constructs are then identified which possess a particular characteristic while maintaining RNAi activity, and can be further modified and assayed once again. This same approach can be used to identify siNA-conjugate molecules with improved pharmacokinetic profiles, delivery, and RNAi activity.

Figure 12 shows non-limiting examples of phosphorylated siNA molecules of the invention, including linear and duplex constructs and asymmetric derivatives thereof.

Figure 13 shows non-limiting examples of chemically modified terminal phosphate groups of the invention.

Figure 14A shows a non-limiting example of methodology used to design self complementary DFO constructs utilizing palidrome and/or repeat nucleic acid sequences that are identified in a target nucleic acid sequence. (i) A palindrome or repeat sequence is identified in a nucleic acid target sequence. (ii) A sequence is designed that is complementary to the target nucleic acid sequence and the palindrome sequence. (iii) An inverse repeat sequence of the non-palindrome/repeat portion of the complementary sequence is appended to the 3'-end of the complementary sequence to generate a self complementary DFO molecule comprising sequence complementary to the nucleic acid target. (iv) The DFO molecule can self-assemble to form a double stranded oligonucleotide. **Figure 14B** shows a non-limiting representative example of a duplex forming oligonucleotide sequence. **Figure 14C** shows a non-limiting example of the self assembly schematic of a representative duplex forming oligonucleotide sequence. **Figure 14D** shows a non-limiting example of the self assembly schematic of a representative duplex forming oligonucleotide sequence followed by interaction with a target nucleic acid sequence resulting in modulation of gene expression.

Figure 15 shows a non-limiting example of the design of self complementary DFO constructs utilizing palidrome and/or repeat nucleic acid sequences that are incorporated

into the DFO constructs that have sequence complementary to any target nucleic acid sequence of interest. Incorporation of these palindrome/repeat sequences allow the design of DFO constructs that form duplexes in which each strand is capable of mediating modulation of target gene expression, for example by RNAi. First, the target sequence is identified. A complementary sequence is then generated in which nucleotide or non-nucleotide modifications (shown as X or Y) are introduced into the complementary sequence that generate an artificial palindrome (shown as XYXYXY in the Figure). An inverse repeat of the non-palindrome/repeat complementary sequence is appended to the 3'-end of the complementary sequence to generate a self complementary DFO comprising sequence complementary to the nucleic acid target. The DFO can self-assemble to form a double stranded oligonucleotide.

Figure 16 shows non-limiting examples of multifunctional siNA molecules of the invention comprising two separate polynucleotide sequences that are each capable of mediating RNAi directed cleavage of differing target nucleic acid sequences. **Figure 16A** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first and second complementary regions are situated at the 3'-ends of each polynucleotide sequence in the multifunctional siNA. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. **Figure 16B** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first and second complementary regions are situated at the 5'-ends of each polynucleotide sequence in the multifunctional siNA. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences.

Figure 17 shows non-limiting examples of multifunctional siNA molecules of the invention comprising a single polynucleotide sequence comprising distinct regions that

are each capable of mediating RNAi directed cleavage of differing target nucleic acid sequences. **Figure 17A** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the second complementary region is situated at the 3'-end of the polynucleotide sequence in the multifunctional siNA. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. **Figure 17B** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first complementary region is situated at the 5'-end of the polynucleotide sequence in the multifunctional siNA. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. In one embodiment, these multifunctional siNA constructs are processed in vivo or in vitro to generate multifunctional siNA constructs as shown in **Figure 16**.

Figure 18 shows non-limiting examples of multifunctional siNA molecules of the invention comprising two separate polynucleotide sequences that are each capable of mediating RNAi directed cleavage of differing target nucleic acid sequences and wherein the multifunctional siNA construct further comprises a self complementary, palindrome, or repeat region, thus enabling shorter bifuctional siNA constructs that can mediate RNA interference against differing target nucleic acid sequences. **Figure 18A** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first and second complementary regions are situated at the 3'-ends of each polynucleotide sequence in the multifunctional siNA, and wherein the first and second complementary regions further comprise a self complementary, palindrome, or repeat region. The dashed portions of each

polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. **Figure 18B** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first and second complementary regions are situated at the 5'-ends of each polynucleotide sequence in the multifunctional siNA, and wherein the first and second complementary regions further comprise a self complementary, palindrome, or repeat region. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences.

Figure 19 shows non-limiting examples of multifunctional siNA molecules of the invention comprising a single polynucleotide sequence comprising distinct regions that are each capable of mediating RNAi directed cleavage of differing target nucleic acid sequences and wherein the multifunctional siNA construct further comprises a self complementary, palindrome, or repeat region, thus enabling shorter bifuctional siNA constructs that can mediate RNA interference against differing target nucleic acid sequences. **Figure 19A** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the second complementary region is situated at the 3'-end of the polynucleotide sequence in the multifunctional siNA, and wherein the first and second complementary regions further comprise a self complementary, palindrome, or repeat region. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. **Figure 19B** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first complementary region is situated at the 5'-

end of the polynucleotide sequence in the multifunctional siNA, and wherein the first and second complementary regions further comprise a self complementary, palindrome, or repeat region. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. In one embodiment, these multifunctional siNA constructs are processed in vivo or in vitro to generate multifunctional siNA constructs as shown in **Figure 18**.

Figure 20 shows a non-limiting example of how multifunctional siNA molecules of the invention can target two separate target nucleic acid molecules, such as separate RNA molecules encoding differing proteins, for example, a cytokine and its corresponding receptor, differing viral strains, a virus and a cellular protein involved in viral infection or replication, or differing proteins involved in a common or divergent biologic pathway that is implicated in the maintenance of progression of disease. Each strand of the multifunctional siNA construct comprises a region having complementarity to separate target nucleic acid molecules. The multifunctional siNA molecule is designed such that each strand of the siNA can be utilized by the RISC complex to initiate RNA interference mediated cleavage of its corresponding target. These design parameters can include destabilization of each end of the siNA construct (see for example Schwarz *et al.*, 2003, *Cell*, 115, 199-208). Such destabilization can be accomplished for example by using guanosine-cytidine base pairs, alternate base pairs (e.g., wobbles), or destabilizing chemically modified nucleotides at terminal nucleotide positions as is known in the art.

Figure 21 shows a non-limiting example of how multifunctional siNA molecules of the invention can target two separate target nucleic acid sequences within the same target nucleic acid molecule, such as alternate coding regions of a RNA, coding and non-coding regions of a RNA, or alternate splice variant regions of a RNA. Each strand of the multifunctional siNA construct comprises a region having complementarity to the separate regions of the target nucleic acid molecule. The multifunctional siNA molecule is designed such that each strand of the siNA can be utilized by the RISC complex to initiate RNA interference mediated cleavage of its corresponding target region. These design parameters can include destabilization of each end of the siNA construct (see for example Schwarz *et al.*, 2003, *Cell*, 115, 199-208). Such destabilization can be

accomplished for example by using guanosine-cytidine base pairs, alternate base pairs (e.g., wobbles), or destabilizing chemically modified nucleotides at terminal nucleotide positions as is known in the art.

Figure 22 shows a non-limiting example of reduction of BACE mRNA levels in A549 cells after treatment with siNA molecules targeting BACE mRNA. A549 cells were transfected with 0.25 ug/well of lipid complexed with 25 nM siNA. A screen of siNA constructs comprising ribonucleotides and 3'-terminal dithymidine caps was compared to untreated cells, scrambled siNA control constructs (Scram 1 and Scram 2), and the cells transfected with lipid alone (transfection control). As shown in the Figure, all of the siNA constructs show significant reduction of BACE RNA expression.

Figure 23 shows a non-limiting example of reduction of BACE mRNA levels in A549 cells (5,000 cells/well) 24 hours after treatment with siNA molecules targeting BACE mRNA. A549 cells were transfected with 0.25 ug/well of lipid complexed with 25 nM siNA. A lead siNA construct (31007/31083) chosen from the screen described in **Figure 22** was further modified using chemical modifications described in **Table IV** herein. Chemically modified constructs having Stab 4/5 chemistry (31378/31381) and Stab 7/11 chemistry (31384/31387) (solid bars; see **Tables III and IV**) were tested for efficacy compared to matched chemistry inverted controls (open bars; sequences shown in **Table III**). The original lead siNA construct (31007/31083) and the Stab 4/5 and Stab 7/11 constructs were compared to untreated cells, scrambled siNA control constructs (Scram1 and Scram2), and cells transfected with lipid alone (transfection control). As shown in the figure, the original lead construct and the Stab 4/5 and Stab 7/11 modified siNA constructs all show significant reduction of BACE RNA expression.

Figure 24 shows a non-limiting example of reduction of APP mRNA in SK-N-SH cells mediated by chemically modified siNAs that target APP mRNA. SK-N-SH cells were transfected with 0.25 ug/well of lipid complexed with 25 nM siNA. Active siNA constructs comprising various stabilization chemistries (solid bars; see **Tables III and IV**) were compared to untreated cells, matched chemistry irrelevant siNA control constructs (IC1), and cells transfected with lipid alone (transfection control). As shown in the figure, the siNA constructs significantly reduce APP RNA expression.

Figure 25 shows a non-limiting example of reduction of PSEN1 mRNA in SK-N-SH cells mediated by chemically modified siNAs that target PSEN1 mRNA. SK-N-SH cells were transfected with 0.25 ug/well of lipid complexed with 25 nM siNA. Active siNA constructs comprising various stabilization chemistries (solid bars; see **Tables III and IV**) were compared to untreated cells, matched chemistry irrelevant siNA control constructs (IC1), and cells transfected with lipid alone (transfection control). As shown in the figure, the siNA constructs significantly reduce PSEN1 RNA expression.

Figure 26 shows a non-limiting example of reduction of PSEN2 mRNA in SK-N-SH cells mediated by chemically modified siNAs that target PSEN2 mRNA. SK-N-SH cells were transfected with 0.25 ug/well of lipid complexed with 25 nM siNA. Active siNA constructs comprising various stabilization chemistries (solid bars; see **Tables III and IV**) were compared to untreated cells, matched chemistry irrelevant siNA control constructs (IC1), and cells transfected with lipid alone (transfection control). As shown in the figure, the siNA constructs significantly reduce PSEN2 RNA expression.

DETAILED DESCRIPTION OF THE INVENTION

Mechanism of Action of Nucleic Acid Molecules of the Invention

The discussion that follows discusses the proposed mechanism of RNA interference mediated by short interfering RNA as is presently known, and is not meant to be limiting and is not an admission of prior art. Applicant demonstrates herein that chemically-modified short interfering nucleic acids possess similar or improved capacity to mediate RNAi as do siRNA molecules and are expected to possess improved stability and activity *in vivo*; therefore, this discussion is not meant to be limiting only to siRNA and can be applied to siNA as a whole. By "improved capacity to mediate RNAi" or "improved RNAi activity" is meant to include RNAi activity measured *in vitro* and/or *in vivo* where the RNAi activity is a reflection of both the ability of the siNA to mediate RNAi and the stability of the siNAs of the invention. In this invention, the product of these activities can be increased *in vitro* and/or *in vivo* compared to an all RNA siRNA or a siNA containing a plurality of ribonucleotides. In some cases, the activity or stability of the siNA molecule can be decreased (i.e., less than ten-fold), but the overall activity of the siNA molecule is enhanced *in vitro* and/or *in vivo*.

RNA interference refers to the process of sequence specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs) (Fire *et al.*, 1998, *Nature,* 391, 806). The corresponding process in plants is commonly referred to as post-transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of post-transcriptional gene silencing is thought to be an evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes which is commonly shared by diverse flora and phyla (Fire *et al.*, 1999, *Trends Genet.*, 15, 358). Such protection from foreign gene expression may have evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA. The presence of dsRNA in cells triggers the RNAi response though a mechanism that has yet to be fully characterized. This mechanism appears to be different from the interferon response that results from dsRNA-mediated activation of protein kinase PKR and 2', 5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L.

The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as Dicer. Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs) (Berstein *et al.*, 2001, *Nature,* 409, 363). Short interfering RNAs derived from Dicer activity are typically about 21 to about 23 nucleotides in length and comprise about 19 base pair duplexes. Dicer has also been implicated in the excision of 21- and 22-nucleotide small temporal RNAs (stRNAs) from precursor RNA of conserved structure that are implicated in translational control (Hutvagner *et al.*, 2001, *Science,* 293, 834). The RNAi response also features an endonuclease complex containing a siRNA, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded RNA having sequence homologous to the siRNA. Cleavage of the target RNA takes place in the middle of the region complementary to the guide sequence of the siRNA duplex (Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188). In addition, RNA interference can also involve small RNA (e.g., micro-RNA or miRNA) mediated gene silencing, presumably though cellular mechanisms that regulate chromatin structure and thereby prevent transcription of target gene sequences (see for example Allshire, 2002, *Science,*

297, 1818-1819; Volpe *et al.*, 2002, *Science,* 297, 1833-1837; Jenuwein, 2002, *Science,* 297, 2215-2218; and Hall *et al.*, 2002, *Science,* 297, 2232-2237). As such, siNA molecules of the invention can be used to mediate gene silencing via interaction with RNA transcripts or alternately by interaction with particular gene sequences, wherein such interaction results in gene silencing either at the transcriptional level or post-transcriptional level.

RNAi has been studied in a variety of systems. Fire *et al.*, 1998, *Nature,* 391, 806, were the first to observe RNAi in *C. elegans*. Wianny and Goetz, 1999, *Nature Cell Biol.*, 2, 70, describe RNAi mediated by dsRNA in mouse embryos. Hammond *et al.*, 2000, *Nature,* 404, 293, describe RNAi in *Drosophila* cells transfected with dsRNA. Elbashir *et al.*, 2001, *Nature,* 411, 494, describe RNAi induced by introduction of duplexes of synthetic 21-nucleotide RNAs in cultured mammalian cells including human embryonic kidney and HeLa cells. Recent work in Drosophila embryonic lysates has revealed certain requirements for siRNA length, structure, chemical composition, and sequence that are essential to mediate efficient RNAi activity. These studies have shown that 21 nucleotide siRNA duplexes are most active when containing two 2-nucleotide 3'-terminal nucleotide overhangs. Furthermore, substitution of one or both siRNA strands with 2'-deoxy or 2'-O-methyl nucleotides abolishes RNAi activity, whereas substitution of 3'-terminal siRNA nucleotides with deoxy nucleotides was shown to be tolerated. Mismatch sequences in the center of the siRNA duplex were also shown to abolish RNAi activity. In addition, these studies also indicate that the position of the cleavage site in the target RNA is defined by the 5'-end of the siRNA guide sequence rather than the 3'-end (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877). Other studies have indicated that a 5'-phosphate on the target-complementary strand of a siRNA duplex is required for siRNA activity and that ATP is utilized to maintain the 5'-phosphate moiety on the siRNA (Nykanen *et al.*, 2001, *Cell,* 107, 309); however, siRNA molecules lacking a 5'-phosphate are active when introduced exogenously, suggesting that 5'-phosphorylation of siRNA constructs may occur *in vivo*.

Synthesis of Nucleic Acid Molecules

Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this

invention, small nucleic acid motifs ("small" refers to nucleic acid motifs no more than 100 nucleotides in length, preferably no more than 80 nucleotides in length, and most preferably no more than 50 nucleotides in length; *e.g.*, individual siNA oligonucleotide sequences or siNA sequences synthesized in tandem) are preferably used for exogenous delivery. The simple structure of these molecules increases the ability of the nucleic acid to invade targeted regions of protein and/or RNA structure. Exemplary molecules of the instant invention are chemically synthesized, and others can similarly be synthesized.

Oligonucleotides (*e.g.*, certain modified oligonucleotides or portions of oligonucleotides lacking ribonucleotides) are synthesized using protocols known in the art, for example as described in Caruthers *et al.*, 1992, *Methods in Enzymology* 211, 3-19, Thompson *et al.*, International PCT Publication No. WO 99/54459, Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677-2684, Wincott *et al.*, 1997, *Methods Mol. Bio.*, 74, 59, Brennan *et al.*, 1998, *Biotechnol Bioeng.*, 61, 33-45, and Brennan, U.S. Pat. No. 6,001,311. All of these references are incorporated herein by reference. The synthesis of oligonucleotides makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2 μmol scale protocol with a 2.5 min coupling step for 2'-O-methylated nucleotides and a 45 second coupling step for 2'-deoxy nucleotides or 2'-deoxy-2'-fluoro nucleotides. **Table V** outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 μmol scale can be performed on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) with minimal modification to the cycle. A 33-fold excess (60 μL of 0.11 M = 6.6 μmol) of 2'-O-methyl phosphoramidite and a 105-fold excess of S-ethyl tetrazole (60 μL of 0.25 M = 15 μmol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 22-fold excess (40 μL of 0.11 M = 4.4 μmol) of deoxy phosphoramidite and a 70-fold excess of S-ethyl tetrazole (40 μL of 0.25 M = 10 μmol) can be used in each coupling cycle of deoxy residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include the following: detritylation solution is 3% TCA in

methylene chloride (ABI); capping is performed with 16% *N*-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); and oxidation solution is 16.9 mM I_2, 49 mM pyridine, 9% water in THF (PerSeptive Biosystems, Inc.). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide, 0.05 M in acetonitrile) is used.

Deprotection of the DNA-based oligonucleotides is performed as follows: the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aqueous methylamine (1 mL) at 65 °C for 10 minutes. After cooling to –20 °C, the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H2O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder.

The method of synthesis used for RNA including certain siNA molecules of the invention follows the procedure as described in Usman *et al.*, 1987, *J. Am. Chem. Soc.*, 109, 7845; Scaringe *et al.*, 1990, *Nucleic Acids Res.*, 18, 5433; and Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677-2684 Wincott *et al.*, 1997, *Methods Mol. Bio.*, 74, 59, and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2 µmol scale protocol with a 7.5 min coupling step for alkylsilyl protected nucleotides and a 2.5 min coupling step for 2'-O-methylated nucleotides. **Table V** outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 µmol scale can be done on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) with minimal modification to the cycle. A 33-fold excess (60 µL of 0.11 M = 6.6 µmol) of 2'-O-methyl phosphoramidite and a 75-fold excess of S-ethyl tetrazole (60 µL of 0.25 M = 15 µmol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 66-fold excess (120 µL of 0.11 M = 13.2 µmol) of alkylsilyl (ribo) protected phosphoramidite and a 150-fold excess of S-ethyl tetrazole (120 µL of 0.25 M

= 30 μmol) can be used in each coupling cycle of ribo residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include the following: detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% *N*-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); oxidation solution is 16.9 mM I_2, 49 mM pyridine, 9% water in THF (PerSeptive Biosystems, Inc.). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide0.05 M in acetonitrile) is used.

Deprotection of the RNA is performed using either a two-pot or one-pot protocol. For the two-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aq. methylamine (1 mL) at 65 °C for 10 min. After cooling to –20 °C, the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H2O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder. The base deprotected oligoribonucleotide is resuspended in anhydrous TEA/HF/NMP solution (300 μL of a solution of 1.5 mL N-methylpyrrolidinone, 750 μL TEA and 1 mL TEA•3HF to provide a 1.4 M HF concentration) and heated to 65 °C. After 1.5 h, the oligomer is quenched with 1.5 M NH_4HCO_3.

Alternatively, for the one-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 33% ethanolic methylamine/DMSO: 1/1 (0.8 mL) at 65 °C for 15 minutes. The vial is brought to room temperature TEA•3HF (0.1 mL) is added and the vial is heated at 65 °C for 15 minutes. The sample is cooled at –20 °C and then quenched with 1.5 M NH_4HCO_3.

For purification of the trityl-on oligomers, the quenched NH_4HCO_3 solution is loaded onto a C-18 containing cartridge that had been prewashed with acetonitrile followed by 50 mM TEAA. After washing the loaded cartridge with water, the RNA is detritylated with 0.5% TFA for 13 minutes. The cartridge is then washed again with water, salt exchanged with 1 M NaCl and washed with water again. The oligonucleotide is then eluted with 30% acetonitrile.

The average stepwise coupling yields are typically >98% (Wincott *et al.*, 1995 *Nucleic Acids Res.* 23, 2677-2684). Those of ordinary skill in the art will recognize that the scale of synthesis can be adapted to be larger or smaller than the example described above including but not limited to 96-well format.

Alternatively, the nucleic acid molecules of the present invention can be synthesized separately and joined together post-synthetically, for example, by ligation (Moore *et al.*, 1992, *Science* 256, 9923; Draper *et al.*, International PCT publication No. WO 93/23569; Shabarova *et al.*, 1991, *Nucleic Acids Research* 19, 4247; Bellon *et al.*, 1997, *Nucleosides & Nucleotides*, 16, 951; Bellon *et al.*, 1997, *Bioconjugate Chem.* 8, 204), or by hybridization following synthesis and/or deprotection.

The siNA molecules of the invention can also be synthesized via a tandem synthesis methodology as described in Example 1 herein, wherein both siNA strands are synthesized as a single contiguous oligonucleotide fragment or strand separated by a cleavable linker which is subsequently cleaved to provide separate siNA fragments or strands that hybridize and permit purification of the siNA duplex. The linker can be a polynucleotide linker or a non-nucleotide linker. The tandem synthesis of siNA as described herein can be readily adapted to both multiwell/multiplate synthesis platforms such as 96 well or similarly larger multi-well platforms. The tandem synthesis of siNA as described herein can also be readily adapted to large scale synthesis platforms employing batch reactors, synthesis columns and the like.

A siNA molecule can also be assembled from two distinct nucleic acid strands or fragments wherein one fragment includes the sense region and the second fragment includes the antisense region of the RNA molecule.

The nucleic acid molecules of the present invention can be modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-*C*-allyl, 2'-fluoro, 2'-*O*-methyl, 2'-H (for a review see Usman and Cedergren, 1992, *TIBS* 17, 34; Usman *et al.*, 1994, *Nucleic Acids Symp. Ser.* 31, 163). siNA constructs can be purified by gel electrophoresis using general methods or can be purified by high pressure liquid chromatography (HPLC; see Wincott *et al., supra*, the totality of which is hereby incorporated herein by reference) and re-suspended in water.

In another aspect of the invention, siNA molecules of the invention are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. The recombinant vectors capable of expressing the siNA molecules can be delivered as described herein, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of siNA molecules.

Optimizing Activity of the nucleic acid molecule of the invention.

Chemically synthesizing nucleic acid molecules with modifications (base, sugar and/or phosphate) can prevent their degradation by serum ribonucleases, which can increase their potency (see *e.g.*, Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*, 1990 *Nature* 344, 565; Pieken *et al.*, 1991, *Science* 253, 314; Usman and Cedergren, 1992, *Trends in Biochem. Sci.* 17, 334; Usman *et al.*, International Publication No. WO 93/15187; and Rossi *et al.*, International Publication No. WO 91/03162; Sproat, U.S. Pat. No. 5,334,711; Gold *et al.*, U.S. Pat. No. 6,300,074; and Burgin *et al., supra;* all of which are incorporated by reference herein). All of the above references describe various chemical modifications that can be made to the base, phosphate and/or sugar moieties of the nucleic acid molecules described herein. Modifications that enhance their efficacy in cells, and removal of bases from nucleic acid molecules to shorten oligonucleotide synthesis times and reduce chemical requirements are desired.

There are several examples in the art describing sugar, base and phosphate modifications that can be introduced into nucleic acid molecules with significant enhancement in their nuclease stability and efficacy. For example, oligonucleotides are

modified to enhance stability and/or enhance biological activity by modification with nuclease resistant groups, for example, 2'-amino, 2'-*C*-allyl, 2'-fluoro, 2'-*O*-methyl, 2'-O-allyl, 2'-H, nucleotide base modifications (for a review see Usman and Cedergren, 1992, *TIBS.* 17, 34; Usman *et al.,* 1994, *Nucleic Acids Symp. Ser.* 31, 163; Burgin *et al.,* 1996, *Biochemistry,* 35, 14090). Sugar modification of nucleic acid molecules have been extensively described in the art (see Eckstein *et al., International Publication* PCT No. WO 92/07065; Perrault *et al. Nature,* 1990, *344,* 565-568; Pieken *et al. Science,* 1991, *253,* 314-317; Usman and Cedergren, *Trends in Biochem. Sci.,* 1992, *17,* 334-339; Usman *et al. International Publication* PCT No. WO 93/15187; Sproat, *U.S. Pat.* No. 5,334,711 and Beigelman *et al.,* 1995, *J. Biol. Chem.,* 270, 25702; Beigelman *et al.,* International PCT publication No. WO 97/26270; Beigelman *et al.,* U.S. Pat. No. 5,716,824; Usman *et al.,* U.S. Pat. No. 5,627,053; Woolf *et al.,* International PCT Publication No. WO 98/13526; Thompson *et al.,* USSN 60/082,404 which was filed on April 20, 1998; Karpeisky *et al.,* 1998, *Tetrahedron Lett.,* 39, 1131; Earnshaw and Gait, 1998, *Biopolymers (Nucleic Acid Sciences),* 48, 39-55; Verma and Eckstein, 1998, *Annu. Rev. Biochem.,* 67, 99-134; and Burlina *et al.,* 1997, *Bioorg. Med. Chem.,* 5, 1999-2010; all of the references are hereby incorporated in their totality by reference herein). Such publications describe general methods and strategies to determine the location of incorporation of sugar, base and/or phosphate modifications and the like into nucleic acid molecules without modulating catalysis, and are incorporated by reference herein. In view of such teachings, similar modifications can be used as described herein to modify the siNA nucleic acid molecules of the instant invention so long as the ability of siNA to promote RNAi is cells is not significantly inhibited.

While chemical modification of oligonucleotide internucleotide linkages with phosphorothioate, phosphorodithioate, and/or 5'-methylphosphonate linkages improves stability, excessive modifications can cause some toxicity or decreased activity. Therefore, when designing nucleic acid molecules, the amount of these internucleotide linkages should be minimized. The reduction in the concentration of these linkages should lower toxicity, resulting in increased efficacy and higher specificity of these molecules.

Short interfering nucleic acid (siNA) molecules having chemical modifications that maintain or enhance activity are provided. Such a nucleic acid is also generally more

resistant to nucleases than an unmodified nucleic acid. Accordingly, the *in vitro* and/or *in vivo* activity should not be significantly lowered. In cases in which modulation is the goal, therapeutic nucleic acid molecules delivered exogenously should optimally be stable within cells until translation of the target RNA has been modulated long enough to reduce the levels of the undesirable protein. This period of time varies between hours to days depending upon the disease state. Improvements in the chemical synthesis of RNA and DNA (Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677; Caruthers *et al.*, 1992, *Methods in Enzymology* 211, 3-19 (incorporated by reference herein)) have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability, as described above.

In one embodiment, nucleic acid molecules of the invention include one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) G-clamp nucleotides. A G-clamp nucleotide is a modified cytosine analog wherein the modifications confer the ability to hydrogen bond both Watson-Crick and Hoogsteen faces of a complementary guanine within a duplex, see for example Lin and Matteucci, 1998, *J. Am. Chem. Soc.*, 120, 8531-8532. A single G-clamp analog substitution within an oligonucleotide can result in substantially enhanced helical thermal stability and mismatch discrimination when hybridized to complementary oligonucleotides. The inclusion of such nucleotides in nucleic acid molecules of the invention results in both enhanced affinity and specificity to nucleic acid targets, complementary sequences, or template strands. In another embodiment, nucleic acid molecules of the invention include one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) LNA "locked nucleic acid" nucleotides such as a 2', 4'-C methylene bicyclo nucleotide (see for example Wengel *et al.*, International PCT Publication No. WO 00/66604 and WO 99/14226).

In another embodiment, the invention features conjugates and/or complexes of siNA molecules of the invention. Such conjugates and/or complexes can be used to facilitate delivery of siNA molecules into a biological system, such as a cell. The conjugates and complexes provided by the instant invention can impart therapeutic activity by transferring therapeutic compounds across cellular membranes, altering the pharmacokinetics, and/or modulating the localization of nucleic acid molecules of the invention. The present invention encompasses the design and synthesis of novel conjugates and complexes for the delivery of molecules, including, but not limited to,

small molecules, lipids, cholesterol, phospholipids, nucleosides, nucleotides, nucleic acids, antibodies, toxins, negatively charged polymers and other polymers, for example proteins, peptides, hormones, carbohydrates, polyethylene glycols, or polyamines, across cellular membranes. In general, the transporters described are designed to be used either individually or as part of a multi-component system, with or without degradable linkers. These compounds are expected to improve delivery and/or localization of nucleic acid molecules of the invention into a number of cell types originating from different tissues, in the presence or absence of serum (see Sullenger and Cech, U.S. Pat. No. 5,854,038). Conjugates of the molecules described herein can be attached to biologically active molecules via linkers that are biodegradable, such as biodegradable nucleic acid linker molecules.

The term "biodegradable linker" as used herein, refers to a nucleic acid or non-nucleic acid linker molecule that is designed as a biodegradable linker to connect one molecule to another molecule, for example, a biologically active molecule to a siNA molecule of the invention or the sense and antisense strands of a siNA molecule of the invention. The biodegradable linker is designed such that its stability can be modulated for a particular purpose, such as delivery to a particular tissue or cell type. The stability of a nucleic acid-based biodegradable linker molecule can be modulated by using various chemistries, for example combinations of ribonucleotides, deoxyribonucleotides, and chemically-modified nucleotides, such as 2'-O-methyl, 2'-fluoro, 2'-amino, 2'-O-amino, 2'-C-allyl, 2'-O-allyl, and other 2'-modified or base modified nucleotides. The biodegradable nucleic acid linker molecule can be a dimer, trimer, tetramer or longer nucleic acid molecule, for example, an oligonucleotide of about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides in length, or can comprise a single nucleotide with a phosphorus-based linkage, for example, a phosphoramidate or phosphodiester linkage. The biodegradable nucleic acid linker molecule can also comprise nucleic acid backbone, nucleic acid sugar, or nucleic acid base modifications.

The term "biodegradable" as used herein, refers to degradation in a biological system, for example, enzymatic degradation or chemical degradation.

The term "biologically active molecule" as used herein refers to compounds or molecules that are capable of eliciting or modifying a biological response in a system.

Non-limiting examples of biologically active siNA molecules either alone or in combination with other molecules contemplated by the instant invention include therapeutically active molecules such as antibodies, cholesterol, hormones, antivirals, peptides, proteins, chemotherapeutics, small molecules, vitamins, co-factors, nucleosides, nucleotides, oligonucleotides, enzymatic nucleic acids, antisense nucleic acids, triplex forming oligonucleotides, 2,5-A chimeras, siNA, dsRNA, allozymes, aptamers, decoys and analogs thereof. Biologically active molecules of the invention also include molecules capable of modulating the pharmacokinetics and/or pharmacodynamics of other biologically active molecules, for example, lipids and polymers such as polyamines, polyamides, polyethylene glycol and other polyethers.

The term "phospholipid" as used herein, refers to a hydrophobic molecule comprising at least one phosphorus group. For example, a phospholipid can comprise a phosphorus-containing group and saturated or unsaturated alkyl group, optionally substituted with OH, COOH, oxo, amine, or substituted or unsubstituted aryl groups.

Therapeutic nucleic acid molecules (*e.g.*, siNA molecules) delivered exogenously optimally are stable within cells until reverse transcription of the RNA has been modulated long enough to reduce the levels of the RNA transcript. The nucleic acid molecules are resistant to nucleases in order to function as effective intracellular therapeutic agents. Improvements in the chemical synthesis of nucleic acid molecules described in the instant invention and in the art have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability as described above.

In yet another embodiment, siNA molecules having chemical modifications that maintain or enhance enzymatic activity of proteins involved in RNAi are provided. Such nucleic acids are also generally more resistant to nucleases than unmodified nucleic acids. Thus, *in vitro* and/or *in vivo* the activity should not be significantly lowered.

Use of the nucleic acid-based molecules of the invention will lead to better treatments by affording the possibility of combination therapies (*e.g.*, multiple siNA molecules targeted to different genes; nucleic acid molecules coupled with known small molecule modulators; or intermittent treatment with combinations of molecules,

including different motifs and/or other chemical or biological molecules). The treatment of subjects with siNA molecules can also include combinations of different types of nucleic acid molecules, such as enzymatic nucleic acid molecules (ribozymes), allozymes, antisense, 2,5-A oligoadenylate, decoys, and aptamers.

In another aspect a siNA molecule of the invention comprises one or more 5' and/or a 3'- cap structure, for example, on only the sense siNA strand, the antisense siNA strand, or both siNA strands.

By "cap structure" is meant chemical modifications, which have been incorporated at either terminus of the oligonucleotide (see, for example, Adamic *et al.*, U.S. Pat. No. 5,998,203, incorporated by reference herein). These terminal modifications protect the nucleic acid molecule from exonuclease degradation, and may help in delivery and/or localization within a cell. The cap may be present at the 5'-terminus (5'-cap) or at the 3'-terminal (3'-cap) or may be present on both termini. In non-limiting examples, the 5'-cap includes, but is not limited to, glyceryl, inverted deoxy abasic residue (moiety); 4',5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide, 4'-thio nucleotide; carbocyclic nucleotide; 1,5-anhydrohexitol nucleotide; L-nucleotides; alpha-nucleotides; modified base nucleotide; phosphorodithioate linkage; *threo*-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; acyclic 3,4-dihydroxybutyl nucleotide; acyclic 3,5-dihydroxypentyl nucleotide, 3'-3'-inverted nucleotide moiety; 3'-3'-inverted abasic moiety; 3'-2'-inverted nucleotide moiety; 3'-2'-inverted abasic moiety; 1,4-butanediol phosphate; 3'-phosphoramidate; hexylphosphate; aminohexyl phosphate; 3'-phosphate; 3'-phosphorothioate; phosphorodithioate; or bridging or non-bridging methylphosphonate moiety.

Non-limiting examples of the 3'-cap include, but are not limited to, glyceryl, inverted deoxy abasic residue (moiety), 4', 5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide; 4'-thio nucleotide, carbocyclic nucleotide; 5'-amino-alkyl phosphate; 1,3-diamino-2-propyl phosphate; 3-aminopropyl phosphate; 6-aminohexyl phosphate; 1,2-aminododecyl phosphate; hydroxypropyl phosphate; 1,5-anhydrohexitol nucleotide; L-nucleotide; alpha-nucleotide; modified base nucleotide; phosphorodithioate; *threo*-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; 3,4-dihydroxybutyl nucleotide; 3,5-dihydroxypentyl nucleotide, 5'-5'-inverted nucleotide

moiety; 5'-5'-inverted abasic moiety; 5'-phosphoramidate; 5'-phosphorothioate; 1,4-butanediol phosphate; 5'-amino; bridging and/or non-bridging 5'-phosphoramidate, phosphorothioate and/or phosphorodithioate, bridging or non bridging methylphosphonate and 5'-mercapto moieties (for more details see Beaucage and Iyer, 1993, *Tetrahedron* 49, 1925; incorporated by reference herein).

By the term "non-nucleotide" is meant any group or compound which can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound is abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine and therefore lacks a base at the 1'-position.

An "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably, it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group can be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2 or $N(CH_3)_2$, amino, or SH. The term also includes alkenyl groups that are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably, it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2, halogen, $N(CH_3)_2$, amino, or SH. The term "alkyl" also includes alkynyl groups that have an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably, it is a lower alkynyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2 or $N(CH_3)_2$, amino or SH.

Such alkyl groups can also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group that has at

least one ring having a conjugated pi electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as described above). Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

By "nucleotide" as used herein is as recognized in the art to include natural bases (standard), and modified bases well known in the art. Such bases are generally located at the 1' position of a nucleotide sugar moiety. Nucleotides generally comprise a base, sugar and a phosphate group. The nucleotides can be unmodified or modified at the sugar, phosphate and/or base moiety, (also referred to interchangeably as nucleotide analogs, modified nucleotides, non-natural nucleotides, non-standard nucleotides and other; see, for example, Usman and McSwiggen, *supra*; Eckstein *et al.*, International PCT Publication No. WO 92/07065; Usman *et al.*, International PCT Publication No. WO 93/15187; Uhlman & Peyman, *supra,* all are hereby incorporated by reference herein). There are several examples of modified nucleic acid bases known in the art as summarized by Limbach *et al.*, 1994, *Nucleic Acids Res.* 22, 2183. Some of the non-limiting examples of base modifications that can be introduced into nucleic acid molecules include, inosine, purine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidines (*e.g.*, 5-methylcytidine), 5-alkyluridines (*e.g.*, ribothymidine), 5-halouridine (*e.g.*, 5-bromouridine) or 6-azapyrimidines or 6-alkylpyrimidines (*e.g.* 6-methyluridine), propyne, and others (Burgin *et al.*, 1996, *Biochemistry*, 35, 14090; Uhlman & Peyman, *supra*). By "modified bases" in this aspect is meant nucleotide bases other than adenine, guanine, cytosine and uracil at 1' position or their equivalents.

In one embodiment, the invention features modified siNA molecules, with phosphate backbone modifications comprising one or more phosphorothioate, phosphorodithioate, methylphosphonate, phosphotriester, morpholino, amidate carbamate, carboxymethyl, acetamidate, polyamide, sulfonate, sulfonamide, sulfamate, formacetal, thioformacetal, and/or alkylsilyl, substitutions. For a review of oligonucleotide backbone modifications, see Hunziker and Leumann, 1995, *Nucleic Acid Analogues: Synthesis and Properties,* in *Modern Synthetic Methods,* VCH, 331-417, and Mesmaeker *et al.,* 1994, *Novel Backbone Replacements for Oligonucleotides,* in *Carbohydrate Modifications in Antisense Research,* ACS, 24-39.

By "abasic" is meant sugar moieties lacking a base or having other chemical groups in place of a base at the 1' position, see for example Adamic *et al.,* U.S. Pat. No. 5,998,203.

By "unmodified nucleoside" is meant one of the bases adenine, cytosine, guanine, thymine, or uracil joined to the 1' carbon of β-D-ribo-furanose.

By "modified nucleoside" is meant any nucleotide base which contains a modification in the chemical structure of an unmodified nucleotide base, sugar and/or phosphate. Non-limiting examples of modified nucleotides are shown by Formulae I-VII and/or other modifications described herein.

In connection with 2'-modified nucleotides as described for the present invention, by "amino" is meant 2'-NH_2 or 2'-*O*- NH_2, which can be modified or unmodified. Such modified groups are described, for example, in Eckstein *et al.,* U.S. Pat. No. 5,672,695 and Matulic-Adamic *et al.,* U.S. Pat. No. 6,248,878, which are both incorporated by reference in their entireties.

Various modifications to nucleic acid siNA structure can be made to enhance the utility of these molecules. Such modifications will enhance shelf-life, half-life *in vitro,* stability, and ease of introduction of such oligonucleotides to the target site, *e.g.,* to enhance penetration of cellular membranes, and confer the ability to recognize and bind to targeted cells.

Administration of Nucleic Acid Molecules

A siNA molecule of the invention can be adapted for use to prevent or treat a variety of neurodegenerative diseases, including Alzheimer's disease, dementia, stroke (CVA), or any other trait, disease or condition that is related to or will respond to the levels of BACE in a cell or tissue, alone or in combination with other therapies.

For example, a siNA molecule can comprise a delivery vehicle, including liposomes, for administration to a subject, carriers and diluents and their salts, and/or can be present in pharmaceutically acceptable formulations. Methods for the delivery of nucleic acid molecules are described in Akhtar *et al.*, 1992, *Trends Cell Bio.*, 2, 139; *Delivery Strategies for Antisense Oligonucleotide Therapeutics*, ed. Akhtar, 1995, Maurer *et al.*, 1999, *Mol. Membr. Biol.*, 16, 129-140; Hofland and Huang, 1999, *Handb. Exp. Pharmacol.*, 137, 165-192; and Lee *et al.*, 2000, *ACS Symp. Ser.*, 752, 184-192, all of which are incorporated herein by reference. Beigelman *et al.*, U.S. Pat. No. 6,395,713 and Sullivan *et al.*, PCT WO 94/02595 further describe the general methods for delivery of nucleic acid molecules. These protocols can be utilized for the delivery of virtually any nucleic acid molecule. Nucleic acid molecules can be administered to cells by a variety of methods known to those of skill in the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as biodegradable polymers, hydrogels, cyclodextrins (see for example Gonzalez *et al.*, 1999, *Bioconjugate Chem.*, 10, 1068-1074; Wang *et al.*, International PCT publication Nos. WO 03/47518 and WO 03/46185), poly(lactic-co-glycolic)acid (PLGA) and PLCA microspheres (see for example US Patent 6,447,796 and US Patent Application Publication No. US 2002130430), biodegradable nanocapsules, and bioadhesive microspheres, or by proteinaceous vectors (O'Hare and Normand, International PCT Publication No. WO 00/53722). In another embodiment, the nucleic acid molecules of the invention can also be formulated or complexed with polyethyleneimine and derivatives thereof, such as polyethyleneimine-polyethyleneglycol-N-acetylgalactosamine (PEI-PEG-GAL) or polyethyleneimine-polyethyleneglycol-tri-N-acetylgalactosamine (PEI-PEG-triGAL) derivatives.

In one embodiment, a siNA molecule of the invention is complexed with membrane disruptive agents such as those described in U.S. Patent Application Publication No. 20010007666, incorporated by reference herein in its entirety including the drawings. In another embodiment, the membrane disruptive agent or agents and the

siNA molecule are also complexed with a cationic lipid or helper lipid molecule, such as those lipids described in U.S. Patent No. 6,235,310, incorporated by reference herein in its entirety including the drawings.

In one embodiment, a siNA molecule of the invention is complexed with delivery systems as described in U.S. Patent Application Publication No. 2003077829 and International PCT Publication Nos. WO 00/03683 and WO 02/087541, all incorporated by reference herein in their entirety including the drawings.

In one embodiment, siNA molecules of the invention are formulated or complexed with polyethylenimine (e.g., linear or branched PEI) and/or polyethylenimine derivatives, including for example grafted PEIs such as galactose PEI, cholesterol PEI, antibody derivatized PEI, and polyethylene glycol PEI (PEG-PEI) derivatives thereof (see for example Ogris *et al.*, 2001, *AAPA PharmSci,* 3, 1-11; Furgeson et al., 2003, Bioconjugate Chem., 14, 840-847; Kunath et al., 2002, Phramaceutical Research, 19, 810-817; Choi et al., 2001, Bull. Korean Chem. Soc., 22, 46-52; Bettinger et al., 1999, Bioconjugate Chem., 10, 558-561; Peterson et al., 2002, Bioconjugate Chem., 13, 845-854; Erbacher et al., 1999, Journal of Gene Medicine Preprint, 1, 1-18; Godbey et al., 1999., PNAS USA, 96, 5177-5181; Godbey et al., 1999, Journal of Controlled Release, 60, 149-160; Diebold et al., 1999, Journal of Biological Chemistry, 274, 19087-19094; Thomas and Klibanov, 2002, PNAS USA, 99, 14640-14645; and Sagara, US 6,586,524, incorporated by reference herein.

In one embodiment, a siNA molecule of the invention comprises a bioconjugate, for example a nucleic acid conjugate as described in Vargeese et al., USSN 10/427,160, filed April 30, 2003; US 6,528,631; US 6,335,434; US 6, 235,886; US 6,153,737; US 5,214,136; US 5,138,045, all incorporated by reference herein.

Thus, the invention features a pharmaceutical composition comprising one or more nucleic acid(s) of the invention in an acceptable carrier, such as a stabilizer, buffer, and the like. The polynucleotides of the invention can be administered (*e.g.*, RNA, DNA or protein) and introduced to a subject by any standard means, with or without stabilizers, buffers, and the like, to form a pharmaceutical composition. When it is desired to use a liposome delivery mechanism, standard protocols for formation of liposomes can be

followed. The compositions of the present invention can also be formulated and used as creams, gels, sprays, oils and other suitable compositions for topical, dermal, or transdermal administration as is known in the art.

The present invention also includes pharmaceutically acceptable formulations of the compounds described. These formulations include salts of the above compounds, *e.g.*, acid addition salts, for example, salts of hydrochloric, hydrobromic, acetic acid, and benzene sulfonic acid.

A pharmacological composition or formulation refers to a composition or formulation in a form suitable for administration, *e.g.*, systemic or local administration, into a cell or subject, including for example a human. Suitable forms, in part, depend upon the use or the route of entry, for example oral, transdermal, or by injection. Such forms should not prevent the composition or formulation from reaching a target cell (*i.e.*, a cell to which the negatively charged nucleic acid is desirable for delivery). For example, pharmacological compositions injected into the blood stream should be soluble. Other factors are known in the art, and include considerations such as toxicity and forms that prevent the composition or formulation from exerting its effect.

In one embodiment, the invention features the use of methods to deliver the nucleic acid molecules of the instant invention to the central nervous system and/or peripheral nervous system. Experiments have demonstrated the efficient *in vivo* uptake of nucleic acids by neurons. As an example of local administration of nucleic acids to nerve cells, Sommer *et al.*, 1998, *Antisense Nuc. Acid Drug Dev.*, 8, 75, describe a study in which a 15mer phosphorothioate antisense nucleic acid molecule to c-fos is administered to rats via microinjection into the brain. Antisense molecules labeled with tetramethylrhodamine-isothiocyanate (TRITC) or fluorescein isothiocyanate (FITC) were taken up by exclusively by neurons thirty minutes post-injection. A diffuse cytoplasmic staining and nuclear staining was observed in these cells. As an example of systemic administration of nucleic acid to nerve cells, Epa *et al.*, 2000, *Antisense Nuc. Acid Drug Dev.*, 10, 469, describe an *in vivo* mouse study in which beta-cyclodextrin-adamantane-oligonucleotide conjugates were used to target the p75 neurotrophin receptor in neuronally differentiated PC12 cells. Following a two week course of IP administration, pronounced uptake of p75 neurotrophin receptor antisense was observed in dorsal root

ganglion (DRG) cells. In addition, a marked and consistent down-regulation of p75 was observed in DRG neurons. Additional approaches to the targeting of nucleic acid to neurons are described in Broaddus *et al.*, 1998, *J. Neurosurg.*, 88(4), 734; Karle *et al.*, 1997, *Eur. J. Pharmocol.*, 340(2/3), 153; Bannai *et al.*, 1998, *Brain Research*, 784(1,2), 304; Rajakumar *et al.*, 1997, *Synapse*, 26(3), 199; Wu-pong *et al.*, 1999, *BioPharm*, 12(1), 32; Bannai *et al.*, 1998, *Brain Res. Protoc.*, 3(1), 83; Simantov *et al.*, 1996, *Neuroscience*, 74(1), 39. Nucleic acid molecules of the invention are therefore amenable to delivery to and uptake by cells that express repeat expansion allelic variants for modulation of RE gene expression. The delivery of nucleic acid molecules of the invention, targeting RE is provided by a variety of different strategies. Traditional approaches to CNS delivery that can be used include, but are not limited to, intrathecal and intracerebroventricular administration, implantation of catheters and pumps, direct injection or perfusion at the site of injury or lesion, injection into the brain arterial system, or by chemical or osmotic opening of the blood-brain barrier. Other approaches can include the use of various transport and carrier systems, for example though the use of conjugates and biodegradable polymers. Furthermore, gene therapy approaches, for example as described in Kaplitt *et al.*, US 6,180,613 and Davidson, WO 04/013280, can be used to express nucleic acid molecules in the CNS.

In one embodiment, nucleic acid molecules of the invention are administered to the central nervous system (CNS) or peripheral nervous system (PNS). Experiments have demonstrated the efficient *in vivo* uptake of nucleic acids by neurons. As an example of local administration of nucleic acids to nerve cells, Sommer *et al.*, 1998, *Antisense Nuc. Acid Drug Dev.*, 8, 75, describe a study in which a 15mer phosphorothioate antisense nucleic acid molecule to c-fos is administered to rats via microinjection into the brain. Antisense molecules labeled with tetramethylrhodamine-isothiocyanate (TRITC) or fluorescein isothiocyanate (FITC) were taken up by exclusively by neurons thirty minutes post-injection. A diffuse cytoplasmic staining and nuclear staining was observed in these cells. As an example of systemic administration of nucleic acid to nerve cells, Epa *et al.*, 2000, *Antisense Nuc. Acid Drug Dev.*, 10, 469, describe an *in vivo* mouse study in which beta-cyclodextrin-adamantane-oligonucleotide conjugates were used to target the p75 neurotrophin receptor in neuronally differentiated PC12 cells. Following a two week course of IP administration, pronounced uptake of p75

neurotrophin receptor antisense was observed in dorsal root ganglion (DRG) cells. In addition, a marked and consistent down-regulation of p75 was observed in DRG neurons. Additional approaches to the targeting of nucleic acid to neurons are described in Broaddus *et al.*, 1998, *J. Neurosurg.*, 88(4), 734; Karle *et al.*, 1997, *Eur. J. Pharmocol.*, 340(2/3), 153; Bannai *et al.*, 1998, *Brain Research*, 784(1,2), 304; Rajakumar *et al.*, 1997, *Synapse*, 26(3), 199; Wu-pong *et al.*, 1999, *BioPharm*, 12(1), 32; Bannai *et al.*, 1998, *Brain Res. Protoc.*, 3(1), 83; Simantov *et al.*, 1996, *Neuroscience*, 74(1), 39. Nucleic acid molecules of the invention are therefore amenable to delivery to and uptake by cells in the CNS and/or PNS.

The delivery of nucleic acid molecules of the invention to the CNS is provided by a variety of different strategies. Traditional approaches to CNS delivery that can be used include, but are not limited to, intrathecal and intracerebroventricular administration, implantation of catheters and pumps, direct injection or perfusion at the site of injury or lesion, injection into the brain arterial system, or by chemical or osmotic opening of the blood-brain barrier. Other approaches can include the use of various transport and carrier systems, for example though the use of conjugates and biodegradable polymers. Furthermore, gene therapy approaches, for example as described in Kaplitt *et al.*, US 6,180,613 and Davidson, WO 04/013280, can be used to express nucleic acid molecules in the CNS.

In one embodiment, dermal delivery systems of the invention include, for example, aqueous and nonaqueous gels, creams, multiple emulsions, microemulsions, liposomes, ointments, aqueous and nonaqueous solutions, lotions, aerosols, hydrocarbon bases and powders, and can contain excipients such as solubilizers, permeation enhancers (e.g., fatty acids, fatty acid esters, fatty alcohols and amino acids), and hydrophilic polymers (e.g., polycarbophil and polyvinylpyrolidone). In one embodiment, the pharmaceutically acceptable carrier is a liposome or a transdermal enhancer. Examples of liposomes which can be used in this invention include the following: (1) CellFectin, 1:1.5 (M/M) liposome formulation of the cationic lipid N,NI,NII,NIII-tetramethyl-N,NI,NII,NIII-tetrapalmit-y-spermine and dioleoyl phosphatidylethanolamine (DOPE) (GIBCO BRL); (2) Cytofectin GSV, 2:1 (M/M) liposome formulation of a cationic lipid and DOPE (Glen Research); (3) DOTAP (N-[1-(2,3-dioleoyloxy)-N,N,N-tri-methyl-ammoniummethylsulfate)

(Boehringer Manheim); and (4) Lipofectamine, 3:1 (M/M) liposome formulation of the polycationic lipid DOSPA and the neutral lipid DOPE (GIBCO BRL).

In one embodiment, siNA molecules of the invention are administered to a subject by systemic administration in a pharmaceutically acceptable composition or formulation. By "systemic administration" is meant *in vivo* systemic absorption or accumulation of drugs in the blood stream followed by distribution throughout the entire body. Administration routes that lead to systemic absorption include, without limitation: intravenous, subcutaneous, intraperitoneal, inhalation, oral, intrapulmonary and intramuscular. Each of these administration routes exposes the siNA molecules of the invention to an accessible diseased tissue. The rate of entry of a drug into the circulation has been shown to be a function of molecular weight or size. The use of a liposome or other drug carrier comprising the compounds of the instant invention can potentially localize the drug, for example, in certain tissue types, such as the tissues of the reticular endothelial system (RES). A liposome formulation that can facilitate the association of drug with the surface of cells, such as, lymphocytes and macrophages is also useful. This approach can provide enhanced delivery of the drug to target cells by taking advantage of the specificity of macrophage and lymphocyte immune recognition of abnormal cells.

In one embodiment, siNA molecules of the invention are formulated or complexed with polyethylenimine (e.g., linear or branched PEI) and/or polyethylenimine derivatives, including for example grafted PEIs such as galactose PEI, cholesterol PEI, antibody derivatized PEI, and polyethylene glycol PEI (PEG-PEI) derivatives thereof (see for example Ogris *et al.*, 2001, *AAPA PharmSci*, 3, 1-11; Furgeson et al., 2003, Bioconjugate Chem., 14, 840-847; Kunath et al., 2002, Phramaceutical Research, 19, 810-817; Choi et al., 2001, Bull. Korean Chem. Soc., 22, 46-52; Bettinger et al., 1999, Bioconjugate Chem., 10, 558-561; Peterson et al., 2002, Bioconjugate Chem., 13, 845-854; Erbacher et al., 1999, Journal of Gene Medicine Preprint, 1, 1-18; Godbey et al., 1999., PNAS USA, 96, 5177-5181; Godbey et al., 1999, Journal of Controlled Release, 60, 149-160; Diebold et al., 1999, Journal of Biological Chemistry, 274, 19087-19094; Thomas and Klibanov, 2002, PNAS USA, 99, 14640-14645; and Sagara, US 6,586,524, incorporated by reference herein.

By "pharmaceutically acceptable formulation" or "pharmaceutically acceptable composition" is meant a composition or formulation that allows for the effective distribution of the nucleic acid molecules of the instant invention in the physical location most suitable for their desired activity. Non-limiting examples of agents suitable for formulation with the nucleic acid molecules of the instant invention include: P-glycoprotein inhibitors (such as Pluronic P85),; biodegradable polymers, such as poly (DL-lactide-coglycolide) microspheres for sustained release delivery (Emerich, DF *et al*, 1999, *Cell Transplant*, 8, 47-58); and loaded nanoparticles, such as those made of polybutylcyanoacrylate. Other non-limiting examples of delivery strategies for the nucleic acid molecules of the instant invention include material described in Boado *et al.*, 1998, *J. Pharm. Sci.*, 87, 1308-1315; Tyler *et al.*, 1999, *FEBS Lett.*, 421, 280-284; Pardridge *et al.*, 1995, *PNAS USA.*, 92, 5592-5596; Boado, 1995, *Adv. Drug Delivery Rev.*, 15, 73-107; Aldrian-Herrada *et al.*, 1998, *Nucleic Acids Res.*, 26, 4910-4916; and Tyler *et al.*, 1999, *PNAS USA.*, 96, 7053-7058.

The invention also features the use of the composition comprising surface-modified liposomes containing poly (ethylene glycol) lipids (PEG-modified, or long-circulating liposomes or stealth liposomes). These formulations offer a method for increasing the accumulation of drugs in target tissues. This class of drug carriers resists opsonization and elimination by the mononuclear phagocytic system (MPS or RES), thereby enabling longer blood circulation times and enhanced tissue exposure for the encapsulated drug (Lasic *et al. Chem. Rev.* 1995, 95, 2601-2627; Ishiwata *et al., Chem. Pharm. Bull.* 1995, 43, 1005-1011). Such liposomes have been shown to accumulate selectively in tumors, presumably by extravasation and capture in the neovascularized target tissues (Lasic *et al., Science* 1995, 267, 1275-1276; Oku *et al.*,1995, *Biochim. Biophys. Acta*, 1238, 86-90). The long-circulating liposomes enhance the pharmacokinetics and pharmacodynamics of DNA and RNA, particularly compared to conventional cationic liposomes which are known to accumulate in tissues of the MPS (Liu *et al., J. Biol. Chem.* 1995, 42, 24864-24870; Choi *et al.*, International PCT Publication No. WO 96/10391; Ansell *et al.*, International PCT Publication No. WO 96/10390; Holland *et al.*, International PCT Publication No. WO 96/10392). Long-circulating liposomes are also likely to protect drugs from nuclease degradation to a

greater extent compared to cationic liposomes, based on their ability to avoid accumulation in metabolically aggressive MPS tissues such as the liver and spleen.

The present invention also includes compositions prepared for storage or administration that include a pharmaceutically effective amount of the desired compounds in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co. (A.R. Gennaro edit. 1985), hereby incorporated by reference herein. For example, preservatives, stabilizers, dyes and flavoring agents can be provided. These include sodium benzoate, sorbic acid and esters of *p*-hydroxybenzoic acid. In addition, antioxidants and suspending agents can be used.

A pharmaceutically effective dose is that dose required to prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state. The pharmaceutically effective dose depends on the type of disease, the composition used, the route of administration, the type of mammal being treated, the physical characteristics of the specific mammal under consideration, concurrent medication, and other factors that those skilled in the medical arts will recognize. Generally, an amount between 0.1 mg/kg and 100 mg/kg body weight/day of active ingredients is administered dependent upon potency of the negatively charged polymer.

The nucleic acid molecules of the invention and formulations thereof can be administered orally, topically, parenterally, by inhalation or spray, or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and/or vehicles. The term parenteral as used herein includes percutaneous, subcutaneous, intravascular (*e.g.*, intravenous), intramuscular, or intrathecal injection or infusion techniques and the like. In addition, there is provided a pharmaceutical formulation comprising a nucleic acid molecule of the invention and a pharmaceutically acceptable carrier. One or more nucleic acid molecules of the invention can be present in association with one or more non-toxic pharmaceutically acceptable carriers and/or diluents and/or adjuvants, and if desired other active ingredients. The pharmaceutical compositions containing nucleic acid molecules of the invention can be in a form suitable for oral use, for example, as tablets, troches,

lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs.

Compositions intended for oral use can be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions can contain one or more such sweetening agents, flavoring agents, coloring agents or preservative agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients that are suitable for the manufacture of tablets. These excipients can be, for example, inert diluents; such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia; and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets can be uncoated or they can be coated by known techniques. In some cases such coatings can be prepared by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monosterate or glyceryl distearate can be employed.

Formulations for oral use can also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

Aqueous suspensions contain the active materials in a mixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydropropyl-methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents can be a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with

partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions can also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions can be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions can contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents and flavoring agents can be added to provide palatable oral preparations. These compositions can be preserved by the addition of an anti-oxidant such as ascorbic acid

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents or suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, can also be present.

Pharmaceutical compositions of the invention can also be in the form of oil-in-water emulsions. The oily phase can be a vegetable oil or a mineral oil or mixtures of these. Suitable emulsifying agents can be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions can also contain sweetening and flavoring agents.

Syrups and elixirs can be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol, glucose or sucrose. Such formulations can also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions can be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension can be formulated according to the known art using those suitable

dispersing or wetting agents and suspending agents that have been mentioned above. The sterile injectable preparation can also be a sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil can be employed including synthetic mono-or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The nucleic acid molecules of the invention can also be administered in the form of suppositories, *e.g.*, for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient that is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include cocoa butter and polyethylene glycols.

Nucleic acid molecules of the invention can be administered parenterally in a sterile medium. The drug, depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as local anesthetics, preservatives and buffering agents can be dissolved in the vehicle.

Dosage levels of the order of from about 0.1 mg to about 140 mg per kilogram of body weight per day are useful in the treatment of the above-indicated conditions (about 0.5 mg to about 7 g per subject per day). The amount of active ingredient that can be combined with the carrier materials to produce a single dosage form varies depending upon the host treated and the particular mode of administration. Dosage unit forms generally contain between from about 1 mg to about 500 mg of an active ingredient.

It is understood that the specific dose level for any particular subject depends upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, and rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

For administration to non-human animals, the composition can also be added to the animal feed or drinking water. It can be convenient to formulate the animal feed and drinking water compositions so that the animal takes in a therapeutically appropriate quantity of the composition along with its diet. It can also be convenient to present the composition as a premix for addition to the feed or drinking water.

The nucleic acid molecules of the present invention can also be administered to a subject in combination with other therapeutic compounds to increase the overall therapeutic effect. The use of multiple compounds to treat an indication can increase the beneficial effects while reducing the presence of side effects.

Alternatively, certain siNA molecules of the instant invention can be expressed within cells from eukaryotic promoters (*e.g.*, Izant and Weintraub, 1985, *Science,* 229, 345; McGarry and Lindquist, 1986, *Proc. Natl. Acad. Sci.*, USA 83, 399; Scanlon *et al.*, 1991, *Proc. Natl. Acad. Sci. USA*, 88, 10591-5; Kashani-Sabet *et al.*, 1992, *Antisense Res. Dev.*, 2, 3-15; Dropulic *et al.*, 1992, *J. Virol.*, 66, 1432-41; Weerasinghe *et al.*, 1991, *J. Virol.*, 65, 5531-4; Ojwang *et al.*, 1992, *Proc. Natl. Acad. Sci. USA*, 89, 10802-6; Chen *et al.*, 1992, *Nucleic Acids Res.*, 20, 4581-9; Sarver *et al.*, 1990 *Science,* 247, 1222-1225; Thompson *et al.*, 1995, *Nucleic Acids Res.*, 23, 2259; Good *et al.*, 1997, *Gene Therapy*, 4, 45. Those skilled in the art realize that any nucleic acid can be expressed in eukaryotic cells from the appropriate DNA/RNA vector. The activity of such nucleic acids can be augmented by their release from the primary transcript by a enzymatic nucleic acid (Draper *et al.*, PCT WO 93/23569, and Sullivan *et al.*, PCT WO 94/02595; Ohkawa *et al.*, 1992, *Nucleic Acids Symp. Ser.*, 27, 15-6; Taira *et al.*, 1991, *Nucleic Acids Res.*, 19, 5125-30; Ventura *et al.*, 1993, *Nucleic Acids Res.*, 21, 3249-55; Chowrira *et al.*, 1994, *J. Biol. Chem.*, 269, 25856.

In another aspect of the invention, RNA molecules of the present invention can be expressed from transcription units (see for example Couture *et al.*, 1996, *TIG.*, 12, 510) inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. In another embodiment, pol III based constructs are used to express nucleic acid molecules of the invention (see for example Thompson, U.S. Pats. Nos. 5,902,880 and 6,146,886). The recombinant

vectors capable of expressing the siNA molecules can be delivered as described above, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of nucleic acid molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the siNA molecule interacts with the target mRNA and generates an RNAi response. Delivery of siNA molecule expressing vectors can be systemic, such as by intravenous or intra-muscular administration, by administration to target cells ex-planted from a subject followed by reintroduction into the subject, or by any other means that would allow for introduction into the desired target cell (for a review see Couture *et al.*, 1996, *TIG.*, 12, 510).

In one aspect the invention features an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the instant invention. The expression vector can encode one or both strands of a siNA duplex, or a single self-complementary strand that self hybridizes into a siNA duplex. The nucleic acid sequences encoding the siNA molecules of the instant invention can be operably linked in a manner that allows expression of the siNA molecule (see for example Paul *et al.*, 2002, *Nature Biotechnology,* 19, 505; Miyagishi and Taira, 2002, *Nature Biotechnology,* 19, 497; Lee *et al.*, 2002, *Nature Biotechnology,* 19, 500; and Novina *et al.*, 2002, *Nature Medicine*, advance online publication doi:10.1038/nm725).

In another aspect, the invention features an expression vector comprising: a) a transcription initiation region (*e.g.*, eukaryotic pol I, II or III initiation region); b) a transcription termination region (*e.g.*, eukaryotic pol I, II or III termination region); and c) a nucleic acid sequence encoding at least one of the siNA molecules of the instant invention, wherein said sequence is operably linked to said initiation region and said termination region in a manner that allows expression and/or delivery of the siNA molecule. The vector can optionally include an open reading frame (ORF) for a protein operably linked on the 5' side or the 3'-side of the sequence encoding the siNA of the invention; and/or an intron (intervening sequences).

Transcription of the siNA molecule sequences can be driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters are expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type depends on the nature

of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990, *Proc. Natl. Acad. Sci. U S A*, 87, 6743-7; Gao and Huang 1993, *Nucleic Acids Res.*, 21, 2867-72; Lieber *et al.*, 1993, *Methods Enzymol.*, 217, 47-66; Zhou *et al.*, 1990, *Mol. Cell. Biol.*, 10, 4529-37). Several investigators have demonstrated that nucleic acid molecules expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet *et al.*, 1992, *Antisense Res. Dev.*, 2, 3-15; Ojwang *et al.*, 1992, *Proc. Natl. Acad. Sci. U S A*, 89, 10802-6; Chen *et al.*, 1992, *Nucleic Acids Res.*, 20, 4581-9; Yu *et al.*, 1993, *Proc. Natl. Acad. Sci. U S A*, 90, 6340-4; L'Huillier *et al.*, 1992, *EMBO J.*, 11, 4411-8; Lisziewicz *et al.*, 1993, *Proc. Natl. Acad. Sci. U. S. A*, 90, 8000-4; Thompson *et al.*, 1995, *Nucleic Acids Res.*, 23, 2259; Sullenger & Cech, 1993, *Science*, 262, 1566). More specifically, transcription units such as the ones derived from genes encoding U6 small nuclear (snRNA), transfer RNA (tRNA) and adenovirus VA RNA are useful in generating high concentrations of desired RNA molecules such as siNA in cells (Thompson *et al., supra*; Couture and Stinchcomb, *1996, supra*; Noonberg *et al.*, 1994, *Nucleic Acid Res.*, 22, 2830; Noonberg *et al.*, U.S. Pat. No. 5,624,803; Good *et al.*, 1997, *Gene Ther.*, 4, 45; Beigelman *et al.*, International PCT Publication No. WO 96/18736. The above siNA transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors) (for a review see Couture and Stinchcomb, *1996, supra).*

In another aspect the invention features an expression vector comprising a nucleic acid sequence encoding at least one of the siNA molecules of the invention in a manner that allows expression of that siNA molecule. The expression vector comprises in one embodiment; a) a transcription initiation region; b) a transcription termination region; and c) a nucleic acid sequence encoding at least one strand of the siNA molecule, wherein the sequence is operably linked to the initiation region and the termination region in a manner that allows expression and/or delivery of the siNA molecule.

In another embodiment the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an open reading frame; and d) a

nucleic acid sequence encoding at least one strand of a siNA molecule, wherein the sequence is operably linked to the 3'-end of the open reading frame and wherein the sequence is operably linked to the initiation region, the open reading frame and the termination region in a manner that allows expression and/or delivery of the siNA molecule. In yet another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; and d) a nucleic acid sequence encoding at least one siNA molecule, wherein the sequence is operably linked to the initiation region, the intron and the termination region in a manner which allows expression and/or delivery of the nucleic acid molecule.

In another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; d) an open reading frame; and e) a nucleic acid sequence encoding at least one strand of a siNA molecule, wherein the sequence is operably linked to the 3'-end of the open reading frame and wherein the sequence is operably linked to the initiation region, the intron, the open reading frame and the termination region in a manner which allows expression and/or delivery of the siNA molecule.

BACE, APP, PIN-1, and PS Biology and Biochemistry

Alzheimer's disease is characterized by the progressive formation of insoluble plaques and vascular deposits in the brain consisting of the 4 kD amyloid β peptide (Aβ). These plaques are characterized by dystrophic neurites that show profound synaptic loss, neurofibrillary tangle formation, and gliosis. Aβ arises from the proteolytic cleavage of the large type I transmembrane protein, β-amyloid precursor protein (APP) (Kang *et al.*, 1987, *Nature,* 325, 733). Processing of APP to generate Aβ requires two sites of cleavage by a β-secretase and a γ-secretase. β-secretase cleavage of APP results in the cytoplasmic release of a 100 kD soluble amino-terminal fragment, APPsβ, leaving behind a 12 kD transmembrane carboxy-terminal fragment, C99. Alternately, APP can be cleaved by a α-secretase to generate cytoplasmic APPsα and transmembrane C83 fragments. Both remaining transmembrane fragments, C99 and C83, can be further cleaved by a γ-secretase, leading to the release and secretion of Alzheimer's related Aβ and a non-pathogenic peptide, p3, respectively (Vassar *et al.*, 1999, *Science,* 286, 735-741). Early onset familial Alzheimer's disease is characterized by mutant APP protein

with a Met to Leu substitution at position P1, characterized as the "Swedish" familial mutation (Mullan *et al.*, 1992, *Nature Genet.*, 1, 345). This APP mutation is characterized by a dramatic enhancement in β-secretase cleavage (Citron *et al.*, 1992, *Nature*, 360, 672).

The identification of β-secretase and γ-secretase constituents involved in the release of β-amyloid protein is of primary importance in the development of treatment strategies for Alzheimer's disease. Characterization of α-secretase is also important in this regard since α-secretase cleavage may compete with β-secretase cleavage resulting in changes in the relative amounts of non-pathogenic and pathogenic protein production. Involvement of the two metalloproteases, ADAM 10 and TACE, has been demonstrated in α-cleavage of AAP (Buxbaum *et al.*, 1999, *J. Biol. Chem.*, 273, 27765, and Lammich *et al.*, 1999, *Proc. Natl. Acad. Sci. U.S.A.*, 96, 3922). Studies of γ-secretase activity have demonstrated presenilin dependence (De Stooper *et al.*, 1998, *Nature*, 391, 387, and De Stooper *et al.*, 1999, *Nature*, 398, 518), and as such, presenilins have been proposed as γ-secretase even though presenilin does not present proteolytic activity (Wolfe *et al.*, 1999, *Nature*, 398, 513).

Studies have shown β-secretase cleavage of AAP by the transmembrane aspartic protease beta site APP cleaving enzyme, BACE (Vassar *et al.*, supra). While other potential candidates for β-secretase have been proposed (for review see Evin *et al.*, 1999, *Proc. Natl. Acad. Sci. U.S.A.*, 96, 3922), none have demonstrated the full range of characteristics expected from this enzyme. Studies have shown that BACE expression and localization are as expected for β-secretase, that BACE overexpression in cells results in increased β-secretase cleavage of APP and Swedish APP, that isolated BACE demonstrates site specific proteolytic activity on APP derived peptide substrates, and that antisense mediated endogenous BACE inhibition results in dramatically reduced β-secretase activity (Vassar *et al.*, supra).

Current treatment strategies for Alzheimer's disease rely on either the prevention or the alleviation of symptoms and/or the slowing down of disease progression. Two drugs approved in the treatment of Alzheimer's, donepezil (Aricept®) and tacrine (Cognex®), both cholinomimetics, attempt to slow the loss of cognitive ability by

increasing the amount of acetylcholine available to the brain. Antioxidant therapy through the use of antioxidant compounds such as alpha-tocopherol (vitamin E), melatonin, and selegeline (Eldepryl®) attempt to slow disease progression by minimizing free radical damage. Estrogen replacement therapy is thought to incur a possible preventative benefit in the development of Alzheimer's disease based on limited data. The use of anti-inflammatory drugs may be associated with a reduced risk of Alzheimer's as well. Calcium channel blockers such as Nimodipine® are considered to have a potential benefit in treating Alzheimer's disease due to protection of nerve cells from calcium overload, thereby prolonging nerve cell survival. Nootropic compounds, such as acetyl-L-carnitine (Alcar®) and insulin, have been proposed to have some benefit in treating Alzheimer's due to enhancement of cognitive and memory function based on cellular metabolism.

Whereby the above treatment strategies can all improve quality of life in Alzheimer's patients, there exists an unmet need in the comprehensive treatment and prevention of this disease. As such, there exists the need for therapeutics effective in reversing the physiological changes associated with Alzheimer's disease, specifically, therapeutics that can eliminate and/or reverse the deposition of amyloid β peptide. The use of compounds,such as small nucleic acid molecules (e.g., short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules capable of mediating RNA interference (RNAi)), to modulate the expression of proteases that are instrumental in the release of amyloid β peptide, namely β-secretase (BACE), γ-secretase (presenilin), and the amyloid precursor protein (APP), is of therapeutic significance.

Examples:

The following are non-limiting examples showing the selection, isolation, synthesis and activity of nucleic acids of the instant invention.

Example 1: Tandem synthesis of siNA constructs

Exemplary siNA molecules of the invention are synthesized in tandem using a cleavable linker, for example, a succinyl-based linker. Tandem synthesis as described herein is followed by a one-step purification process that provides RNAi molecules in

high yield. This approach is highly amenable to siNA synthesis in support of high throughput RNAi screening, and can be readily adapted to multi-column or multi-well synthesis platforms.

After completing a tandem synthesis of a siNA oligo and its complement in which the 5'-terminal dimethoxytrityl (5'-O-DMT) group remains intact (trityl on synthesis), the oligonucleotides are deprotected as described above. Following deprotection, the siNA sequence strands are allowed to spontaneously hybridize. This hybridization yields a duplex in which one strand has retained the 5'-O-DMT group while the complementary strand comprises a terminal 5'-hydroxyl. The newly formed duplex behaves as a single molecule during routine solid-phase extraction purification (Trityl-On purification) even though only one molecule has a dimethoxytrityl group. Because the strands form a stable duplex, this dimethoxytrityl group (or an equivalent group, such as other trityl groups or other hydrophobic moieties) is all that is required to purify the pair of oligos, for example, by using a C18 cartridge.

Standard phosphoramidite synthesis chemistry is used up to the point of introducing a tandem linker, such as an inverted deoxy abasic succinate or glyceryl succinate linker (see **Figure 1**) or an equivalent cleavable linker. A non-limiting example of linker coupling conditions that can be used includes a hindered base such as diisopropylethylamine (DIPA) and/or DMAP in the presence of an activator reagent such as Bromotripyrrolidinophosphoniumhexaflurorophosphate (PyBrOP). After the linker is coupled, standard synthesis chemistry is utilized to complete synthesis of the second sequence leaving the terminal the 5'-O-DMT intact. Following synthesis, the resulting oligonucleotide is deprotected according to the procedures described herein and quenched with a suitable buffer, for example with 50mM NaOAc or 1.5M $NH_4H_2CO_3$.

Purification of the siNA duplex can be readily accomplished using solid phase extraction, for example, using a Waters C18 SepPak 1g cartridge conditioned with 1 column volume (CV) of acetonitrile, 2 CV H2O, and 2 CV 50mM NaOAc. The sample is loaded and then washed with 1 CV H2O or 50mM NaOAc. Failure sequences are eluted with 1 CV 14% ACN (Aqueous with 50mM NaOAc and 50mM NaCl). The column is then washed, for example with 1 CV H2O followed by on-column detritylation, for example by passing 1 CV of 1% aqueous trifluoroacetic acid (TFA)

over the column, then adding a second CV of 1% aqueous TFA to the column and allowing to stand for approximately 10 minutes. The remaining TFA solution is removed and the column washed with H20 followed by 1 CV 1M NaCl and additional H2O. The siNA duplex product is then eluted, for example, using 1 CV 20% aqueous CAN.

Figure 2 provides an example of MALDI-TOF mass spectrometry analysis of a purified siNA construct in which each peak corresponds to the calculated mass of an individual siNA strand of the siNA duplex. The same purified siNA provides three peaks when analyzed by capillary gel electrophoresis (CGE), one peak presumably corresponding to the duplex siNA, and two peaks presumably corresponding to the separate siNA sequence strands. Ion exchange HPLC analysis of the same siNA contract only shows a single peak. Testing of the purified siNA construct using a luciferase reporter assay described below demonstrated the same RNAi activity compared to siNA constructs generated from separately synthesized oligonucleotide sequence strands.

Example 2: Identification of potential siNA target sites in any RNA sequence

The sequence of an RNA target of interest, such as a viral or human mRNA transcript, is screened for target sites, for example by using a computer folding algorithm. In a non-limiting example, the sequence of a gene or RNA gene transcript derived from a database, such as Genbank, is used to generate siNA targets having complementarity to the target. Such sequences can be obtained from a database, or can be determined experimentally as known in the art. Target sites that are known, for example, those target sites determined to be effective target sites based on studies with other nucleic acid molecules, for example ribozymes or antisense, or those targets known to be associated with a disease or condition such as those sites containing mutations or deletions, can be used to design siNA molecules targeting those sites. Various parameters can be used to determine which sites are the most suitable target sites within the target RNA sequence. These parameters include but are not limited to secondary or tertiary RNA structure, the nucleotide base composition of the target sequence, the degree of homology between various regions of the target sequence, or the relative position of the target sequence within the RNA transcript. Based on these determinations, any number of target sites within the RNA transcript can be chosen to

screen siNA molecules for efficacy, for example by using *in vitro* RNA cleavage assays, cell culture, or animal models. In a non-limiting example, anywhere from 1 to 1000 target sites are chosen within the transcript based on the size of the siNA construct to be used. High throughput screening assays can be developed for screening siNA molecules using methods known in the art, such as with multi-well or multi-plate assays to determine efficient reduction in target gene expression.

Example 3: Selection of siNA molecule target sites in a RNA

The following non-limiting steps can be used to carry out the selection of siNAs targeting a given gene sequence or transcript.

1. The target sequence is parsed *in silico* into a list of all fragments or subsequences of a particular length, for example 23 nucleotide fragments, contained within the target sequence. This step is typically carried out using a custom Perl script, but commercial sequence analysis programs such as Oligo, MacVector, or the GCG Wisconsin Package can be employed as well.

2. In some instances the siNAs correspond to more than one target sequence; such would be the case for example in targeting different transcripts of the same gene, targeting different transcripts of more than one gene, or for targeting both the human gene and an animal homolog. In this case, a subsequence list of a particular length is generated for each of the targets, and then the lists are compared to find matching sequences in each list. The subsequences are then ranked according to the number of target sequences that contain the given subsequence; the goal is to find subsequences that are present in most or all of the target sequences. Alternately, the ranking can identify subsequences that are unique to a target sequence, such as a mutant target sequence. Such an approach would enable the use of siNA to target specifically the mutant sequence and not effect the expression of the normal sequence.

3. In some instances the siNA subsequences are absent in one or more sequences while present in the desired target sequence; such would be the case if the siNA targets a gene with a paralogous family member that is to remain untargeted. As in case 2 above, a subsequence list of a particular length is generated for each of the targets,

and then the lists are compared to find sequences that are present in the target gene but are absent in the untargeted paralog.

4. The ranked siNA subsequences can be further analyzed and ranked according to GC content. A preference can be given to sites containing 30-70% GC, with a further preference to sites containing 40-60% GC.

5. The ranked siNA subsequences can be further analyzed and ranked according to self-folding and internal hairpins. Weaker internal folds are preferred; strong hairpin structures are to be avoided.

6. The ranked siNA subsequences can be further analyzed and ranked according to whether they have runs of GGG or CCC in the sequence. GGG (or even more Gs) in either strand can make oligonucleotide synthesis problematic and can potentially interfere with RNAi activity, so it is avoided whenever better sequences are available. CCC is searched in the target strand because that will place GGG in the antisense strand.

7. The ranked siNA subsequences can be further analyzed and ranked according to whether they have the dinucleotide UU (uridine dinucleotide) on the 3'-end of the sequence, and/or AA on the 5'-end of the sequence (to yield 3' UU on the antisense sequence). These sequences allow one to design siNA molecules with terminal TT thymidine dinucleotides.

8. Four or five target sites are chosen from the ranked list of subsequences as described above. For example, in subsequences having 23 nucleotides, the right 21 nucleotides of each chosen 23-mer subsequence are then designed and synthesized for the upper (sense) strand of the siNA duplex, while the reverse complement of the left 21 nucleotides of each chosen 23-mer subsequence are then designed and synthesized for the lower (antisense) strand of the siNA duplex (see **Tables II and III**). If terminal TT residues are desired for the sequence (as described in paragraph 7), then the two 3' terminal nucleotides of both the sense and antisense strands are replaced by TT prior to synthesizing the oligos.

9. The siNA molecules are screened in an *in vitro*, cell culture or animal model system to identify the most active siNA molecule or the most preferred target site within the target RNA sequence.

10. Other design considerations can be used when selecting target nucleic acid sequences, see, for example, Reynolds *et al.*, 2004, *Nature Biotechnology Advanced Online Publication*, 1 February 2004, doi:10.1038/nbt936 and Ui-Tei et al., 2004, Nucleic Acids Research, 32, doi:10.1093/nar/gkh247.

In an alternate approach, a pool of siNA constructs specific to a BACE target sequence is used to screen for target sites in cells expressing BACE RNA, such as cultured A549 cells, 7PA2 cells, Chinese hamster ovary (CHO) cells, or APPsw (Swedish type amyloid precursor protein expressing) cells. The general strategy used in this approach is shown in **Figure 9.** A non-limiting example of such is a pool comprising sequences having any of SEQ ID NOS 1-1900. Cells expressing BACE (e.g., A549 cells) are transfected with the pool of siNA constructs and cells that demonstrate a phenotype associated with BACE inhibition are sorted. The pool of siNA constructs can be expressed from transcription cassettes inserted into appropriate vectors (see for example **Figure 7** and **Figure 8**). The siNA from cells demonstrating a positive phenotypic change (e.g., decreased proliferation, decreased BACE mRNA levels or decreased BACE protein expression), are sequenced to determine the most suitable target site(s) within the target BACE RNA sequence.

Example 4: BACE targeted siNA design

siNA target sites were chosen by analyzing sequences of the BACE RNA target and optionally prioritizing the target sites on the basis of folding (structure of any given sequence analyzed to determine siNA accessibility to the target), by using a library of siNA molecules as described in Example 3, or alternately by using an *in vitro* siNA system as described in Example 6 herein. siNA molecules were designed that could bind each target and are optionally individually analyzed by computer folding to assess whether the siNA molecule can interact with the target sequence. Varying the length of the siNA molecules can be chosen to optimize activity. Generally, a sufficient number of complementary nucleotide bases are chosen to bind to, or otherwise interact with, the target RNA, but the degree of complementarity can be modulated to accommodate siNA

duplexes or varying length or base composition. By using such methodologies, siNA molecules can be designed to target sites within any known RNA sequence, for example those RNA sequences corresponding to the any gene transcript.

Chemically modified siNA constructs are designed to provide nuclease stability for systemic administration in vivo and/or improved pharmacokinetic, localization, and delivery properties while preserving the ability to mediate RNAi activity. Chemical modifications as described herein are introduced synthetically using synthetic methods described herein and those generally known in the art. The synthetic siNA constructs are then assayed for nuclease stability in serum and/or cellular/tissue extracts (e.g. liver extracts). The synthetic siNA constructs are also tested in parallel for RNAi activity using an appropriate assay, such as a luciferase reporter assay as described herein or another suitable assay that can quantity RNAi activity. Synthetic siNA constructs that possess both nuclease stability and RNAi activity can be further modified and re-evaluated in stability and activity assays. The chemical modifications of the stabilized active siNA constructs can then be applied to any siNA sequence targeting any chosen RNA and used, for example, in target screening assays to pick lead siNA compounds for therapeutic development (see for example **Figure 11**).

Example 5: Chemical Synthesis and Purification of siNA

siNA molecules can be designed to interact with various sites in the RNA message, for example, target sequences within the RNA sequences described herein. The sequence of one strand of the siNA molecule(s) is complementary to the target site sequences described above. The siNA molecules can be chemically synthesized using methods described herein. Inactive siNA molecules that are used as control sequences can be synthesized by scrambling the sequence of the siNA molecules such that it is not complementary to the target sequence. Generally, siNA constructs can by synthesized using solid phase oligonucleotide synthesis methods as described herein (see for example Usman *et al.*, US Patent Nos. 5,804,683; 5,831,071; 5,998,203; 6,117,657; 6,353,098; 6,362,323; 6,437,117; 6,469,158; Scaringe *et al.*, US Patent Nos. 6,111,086; 6,008,400; 6,111,086 all incorporated by reference herein in their entirety).

In a non-limiting example, RNA oligonucleotides are synthesized in a stepwise fashion using the phosphoramidite chemistry as is known in the art. Standard

phosphoramidite chemistry involves the use of nucleosides comprising any of 5'-O-dimethoxytrityl, 2'-O-tert-butyldimethylsilyl, 3'-O-2-Cyanoethyl N,N-diisopropylphosphoroamidite groups, and exocyclic amine protecting groups (e.g. N6-benzoyl adenosine, N4 acetyl cytidine, and N2-isobutyryl guanosine). Alternately, 2'-O-Silyl Ethers can be used in conjunction with acid-labile 2'-O-orthoester protecting groups in the synthesis of RNA as described by Scaringe *supra*. Differing 2' chemistries can require different protecting groups, for example 2'-deoxy-2'-amino nucleosides can utilize N-phthaloyl protection as described by Usman *et al.*, US Patent 5,631,360, incorporated by reference herein in its entirety).

During solid phase synthesis, each nucleotide is added sequentially (3'- to 5'-direction) to the solid support-bound oligonucleotide. The first nucleoside at the 3'-end of the chain is covalently attached to a solid support (e.g., controlled pore glass or polystyrene) using various linkers. The nucleotide precursor, a ribonucleoside phosphoramidite, and activator are combined resulting in the coupling of the second nucleoside phosphoramidite onto the 5'-end of the first nucleoside. The support is then washed and any unreacted 5'-hydroxyl groups are capped with a capping reagent such as acetic anhydride to yield inactive 5'-acetyl moieties. The trivalent phosphorus linkage is then oxidized to a more stable phosphate linkage. At the end of the nucleotide addition cycle, the 5'-O-protecting group is cleaved under suitable conditions (e.g., acidic conditions for trityl-based groups and Fluoride for silyl-based groups). The cycle is repeated for each subsequent nucleotide.

Modification of synthesis conditions can be used to optimize coupling efficiency, for example by using differing coupling times, differing reagent/phosphoramidite concentrations, differing contact times, differing solid supports and solid support linker chemistries depending on the particular chemical composition of the siNA to be synthesized. Deprotection and purification of the siNA can be performed as is generally described in Deprotection and purification of the siNA can be performed as is generally described in Usman *et al.*, US 5,831,071, US 6,353,098, US 6,437,117, and Bellon *et al.*, US 6,054,576, US 6,162,909, US 6,303,773, or Scaringe *supra*, incorporated by reference herein in their entireties. Additionally, deprotection conditions can be modified to provide the best possible yield and purity of siNA constructs. For example, applicant has observed that oligonucleotides comprising 2'-deoxy-2'-fluoro nucleotides

can degrade under inappropriate deprotection conditions. Such oligonucleotides are deprotected using aqueous methylamine at about 35°C for 30 minutes. If the 2'-deoxy-2'-fluoro containing oligonucleotide also comprises ribonucleotides, after deprotection with aqueous methylamine at about 35°C for 30 minutes, TEA-HF is added and the reaction maintained at about 65°C for an additional 15 minutes.

Example 6: RNAi *in vitro* assay to assess siNA activity

An *in vitro* assay that recapitulates RNAi in a cell-free system is used to evaluate siNA constructs targeting BACE RNA targets. The assay comprises the system described by Tuschl *et al.*, 1999, *Genes and Development*, 13, 3191-3197 and Zamore *et al.*, 2000, *Cell*, 101, 25-33 adapted for use with BACE target RNA. A Drosophila extract derived from syncytial blastoderm is used to reconstitute RNAi activity *in vitro*. Target RNA is generated via *in vitro* transcription from an appropriate BACE expressing plasmid using T7 RNA polymerase or via chemical synthesis as described herein. Sense and antisense siNA strands (for example 20 uM each) are annealed by incubation in buffer (such as 100 mM potassium acetate, 30 mM HEPES-KOH, pH 7.4, 2 mM magnesium acetate) for 1 minute at 90°C followed by 1 hour at 37°C , then diluted in lysis buffer (for example 100 mM potassium acetate, 30 mM HEPES-KOH at pH 7.4, 2mM magnesium acetate). Annealing can be monitored by gel electrophoresis on an agarose gel in TBE buffer and stained with ethidium bromide. The Drosophila lysate is prepared using zero to two-hour-old embryos from Oregon R flies collected on yeasted molasses agar that are dechorionated and lysed. The lysate is centrifuged and the supernatant isolated. The assay comprises a reaction mixture containing 50% lysate [vol/vol], RNA (10-50 pM final concentration), and 10% [vol/vol] lysis buffer containing siNA (10 nM final concentration). The reaction mixture also contains 10 mM creatine phosphate, 10 ug/ml creatine phosphokinase, 100 um GTP, 100 uM UTP, 100 uM CTP, 500 uM ATP, 5 mM DTT, 0.1 U/uL RNasin (Promega), and 100 uM of each amino acid. The final concentration of potassium acetate is adjusted to 100 mM. The reactions are pre-assembled on ice and preincubated at 25° C for 10 minutes before adding RNA, then incubated at 25° C for an additional 60 minutes. Reactions are quenched with 4 volumes of 1.25 x Passive Lysis Buffer (Promega). Target RNA cleavage is assayed by RT-PCR analysis or other methods known in the art and are compared to control reactions in which siNA is omitted from the reaction.

Alternately, internally-labeled target RNA for the assay is prepared by *in vitro* transcription in the presence of [alpha-^{32}p] CTP, passed over a G50 Sephadex column by spin chromatography and used as target RNA without further purification. Optionally, target RNA is 5'-^{32}P-end labeled using T4 polynucleotide kinase enzyme. Assays are performed as described above and target RNA and the specific RNA cleavage products generated by RNAi are visualized on an autoradiograph of a gel. The percentage of cleavage is determined by PHOSPHOR IMAGER® (autoradiography) quantitation of bands representing intact control RNA or RNA from control reactions without siNA and the cleavage products generated by the assay.

In one embodiment, this assay is used to determine target sites in the BACE RNA target for siNA mediated RNAi cleavage, wherein a plurality of siNA constructs are screened for RNAi mediated cleavage of the BACE RNA target, for example, by analyzing the assay reaction by electrophoresis of labeled target RNA, or by northern blotting, as well as by other methodology well known in the art.

Example 7: Nucleic acid inhibition of BACE target RNA

siNA molecules targeted to the human BACE RNA are designed and synthesized as described above. These nucleic acid molecules can be tested for cleavage activity *in vivo*, for example, using the following procedure. The target sequences and the nucleotide location within the BACE RNA are given in **Tables II and III.**

Two formats are used to test the efficacy of siNAs targeting BACE. First, the reagents are tested in cell culture using, for example, cultured A549 cells, 7PA2 cells, Chinese hamster ovary (CHO) cells, APPsw (Swedish type amyloid precursor protein expressing) cells, or SK-N-SH cells, to determine the extent of RNA and protein inhibition. siNA reagents (*e.g.*; see **Tables II and III**) are selected against the BACE target as described herein. RNA inhibition is measured after delivery of these reagents by a suitable transfection agent to, for example, A549 cells, 7PA2 cells, CHO cells, APPsw cells, or SK-N-SH cells. Relative amounts of target RNA are measured versus actin using real-time PCR monitoring of amplification (*eg.*, ABI 7700 TAQMAN®). A comparison is made to a mixture of oligonucleotide sequences made to unrelated targets or to a randomized siNA control with the same overall length and chemistry, but

randomly substituted at each position. Primary and secondary lead reagents are chosen for the target and optimization performed. After an optimal transfection agent concentration is chosen, a RNA time-course of inhibition is performed with the lead siNA molecule. In addition, a cell-plating format can be used to determine RNA inhibition.

Delivery of siNA to Cells

Cells (e.g., A549 cells, 7PA2 cells, CHO cells, APPsw cells, or SK-N-SH cells) are seeded, for example, at $1x10^5$ cells per well of a six-well dish in EGM-2 (BioWhittaker) the day before transfection. siNA (final concentration, for example 20nM) and cationic lipid (e.g., final concentration 2μg/ml) are complexed in EGM basal media (Bio Whittaker) at 37°C for 30 minutes in polystyrene tubes. Following vortexing, the complexed siNA is added to each well and incubated for the times indicated. For initial optimization experiments, cells are seeded, for example, at $1x10^3$ in 96 well plates and siNA complex added as described. Efficiency of delivery of siNA to cells is determined using a fluorescent siNA complexed with lipid. Cells in 6-well dishes are incubated with siNA for 24 hours, rinsed with PBS and fixed in 2% paraformaldehyde for 15 minutes at room temperature. Uptake of siNA is visualized using a fluorescent microscope.

TAQMAN® (real-time PCR monitoring of amplification) and Lightcycler quantification of mRNA

Total RNA is prepared from cells following siNA delivery, for example, using Qiagen RNA purification kits for 6-well or Rneasy extraction kits for 96-well assays. For TAQMAN® analysis (real-time PCR monitoring of amplification), dual-labeled probes are synthesized with the reporter dye, FAM or JOE, covalently linked at the 5'-end and the quencher dye TAMRA conjugated to the 3'-end. One-step RT-PCR amplifications are performed on, for example, an ABI PRISM 7700 Sequence Detector using 50 μl reactions consisting of 10 μl total RNA, 100 nM forward primer, 900 nM reverse primer, 100 nM probe, 1X TaqMan PCR reaction buffer (PE-Applied Biosystems), 5.5 mM $MgCl_2$, 300 μM each dATP, dCTP, dGTP, and dTTP, 10U RNase Inhibitor (Promega), 1.25U AMPLITAQ GOLD® (DNA polymerase) (PE-Applied Biosystems) and 10U M-MLV Reverse Transcriptase (Promega). The thermal cycling conditions can consist of

30 minutes at 48°C, 10 minutes at 95°C, followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. Quantitation of mRNA levels is determined relative to standards generated from serially diluted total cellular RNA (300, 100, 33, 11 ng/rxn) and normalizing to ß-actin or GAPDH mRNA in parallel TAQMAN® reactions (real-time PCR monitoring of amplification). For each gene of interest an upper and lower primer and a fluorescently labeled probe are designed. Real time incorporation of SYBR Green I dye into a specific PCR product can be measured in glass capillary tubes using a lightcyler. A standard curve is generated for each primer pair using control cRNA. Values are represented as relative expression to GAPDH in each sample.

Western blotting

Nuclear extracts can be prepared using a standard micro preparation technique (see for example Andrews and Faller, 1991, *Nucleic Acids Research,* 19, 2499). Protein extracts from supernatants are prepared, for example using TCA precipitation. An equal volume of 20% TCA is added to the cell supernatant, incubated on ice for 1 hour and pelleted by centrifugation for 5 minutes. Pellets are washed in acetone, dried and resuspended in water. Cellular protein extracts are run on a 10% Bis-Tris NuPage (nuclear extracts) or 4-12% Tris-Glycine (supernatant extracts) polyacrylamide gel and transferred onto nitro-cellulose membranes. Non-specific binding can be blocked by incubation, for example, with 5% non-fat milk for 1 hour followed by primary antibody for 16 hour at 4°C. Following washes, the secondary antibody is applied, for example (1:10,000 dilution) for 1 hour at room temperature and the signal detected with SuperSignal reagent (Pierce).

Example 8: Models useful to evaluate the down-regulation of BACE gene expression

Cell Culture

Vassar *et al.,* 1999, *Science,* 286, 735-741, describe a cell culture model for studying BACE inhibition. Specific antisense nucleic acid molecules targeting BACE mRNA were used for inhibition studies of endogenous BACE expression in 101 cells and APPsw (Swedish type amyloid precursor protein expressing) cells via lipid mediated transfection. Antisense treatment resulted in dramatic reduction of both BACE mRNA by Northern blot analysis, and APPsβsw ("Swedish" type β-secretase cleavage product)

by ELISA, with maximum inhibition of both parameters at 75-80%. This model was also used to study the effect of BACE inhibition on amyloid β-peptide production in APPsw cells. Similarly, such a model can be used to screen siRNA molecules of the instant invention for efficacy and potency.

In several cell culture systems, cationic lipids have been shown to enhance the bioavailability of oligonucleotides to cells in culture (Bennet, *et al.*, 1992, *Mol. Pharmacology*, 41, 1023-1033). In one embodiment, siNA molecules of the invention are complexed with cationic lipids for cell culture experiments. siNA and cationic lipid mixtures are prepared in serum-free DMEM immediately prior to addition to the cells. DMEM plus additives are warmed to room temperature (about 20-25°C) and cationic lipid is added to the final desired concentration and the solution is vortexed briefly. siNA molecules are added to the final desired concentration and the solution is again vortexed briefly and incubated for 10 minutes at room temperature. In dose response experiments, the RNA/lipid complex is serially diluted into DMEM following the 10 minute incubation.

Animal Models

Evaluating the efficacy of anti-BACE agents in animal models is an important prerequisite to human clinical trials. Games *et al.*, 1995, *Nature*, 373, 523-527, describe a transgenic mouse model in which mutant human familial type APP (Phe 717 instead of Val) is overexpressed. This model results in mice that progressively develop many of the pathological hallmarks of Alzheimer's disease, and as such, provides a model for testing therapeutic drugs, including siNA constructs of the instant invention.

Example 9: RNAi mediated inhibition of BACE, APP, PS1 or PS2 expression in cell culture

Inhibition of BACE, APP, PS1, or PS2 RNA expression using siNA targeting BACE, APP, PS1, or PS2 RNA

siNA constructs (**Table III**) are tested for efficacy in reducing BACE, APP, PS1 or PS2 RNA expression in A549 or SK-N-SH cells. Cells are plated approximately 24 hours before transfection in 96-well plates at 5,000-7,500 cells/well, 100 µl/well, such

that at the time of transfection cells are 70-90% confluent. For transfection, annealed siNAs are mixed with the transfection reagent (Lipofectamine 2000, Invitrogen) in a volume of 50 µl/well and incubated for 20 minutes at room temperature. The siNA transfection mixtures are added to cells to give a final siNA concentration of 25 nM in a volume of 150 µl. Each siNA transfection mixture is added to 3 wells for triplicate siNA treatments. Cells are incubated at 37° for 24 hours in the continued presence of the siNA transfection mixture. At 24 hours, RNA is prepared from each well of treated cells. The supernatants with the transfection mixtures are first removed and discarded, then the cells are lysed and RNA prepared from each well. Target gene expression following treatment is evaluated by RT-PCR for the target gene and for a control gene (36B4, an RNA polymerase subunit) for normalization. The triplicate data is averaged and the standard deviations determined for each treatment. Normalized data are graphed and the percent reduction of target mRNA by active siNAs in comparison to their respective inverted control siNAs is determined.

In a non-limiting example, using the method described above, siNA constructs were screened for activity (see **Figure 22**) and compared to untreated cells, scrambled siNA control constructs (Scram1 and Scram2), and cells transfected with lipid alone (transfection control). As shown in **Figure 22**, the siNA constructs show significant reduction of BACE RNA expression. Leads generated from such a screen are then further assayed. In a non-limiting example, siNA constructs comprising ribonucleotides and 3'-terminal dithymidine caps are assayed along with a chemically modified siNA construct comprising 2'-deoxy-2'-fluoro pyrimidine nucleotides and purine ribonucleotides, in which the sense strand of the siNA is further modified with 5' and 3'-terminal inverted deoxyabasic caps and the antisense strand comprises a 3'-terminal phosphorothioate internucleotide linkage. Additional stabilization chemistries as described in **Table IV** are similarly assayed for activity. These siNA constructs are compared to appropriate matched chemistry inverted controls. In addition, the siNA constructs are also compared to untreated cells, cells transfected with lipid and scrambled siNA constructs, and cells transfected with lipid alone (transfection control).

Using the method described above, a lead siNA construct (31007/31083) chosen from the screen described in **Figure 22** above was further modified using chemical modifications described in **Table IV** herein. Results are shown in **Figure 23**. A549

cells were transfected with 0.25 ug/well of lipid complexed with 25 nM siNA. Chemically modified constructs having Stab 4/5 chemistry (31378/31381) and Stab 7/11 chemistry (31384/31387) (solid bars; see **Table IV**) were tested for efficacy compared to matched chemistry inverted controls (open bars; sequences of the siNA constructs shown in **Table III**). The original lead siNA construct (31007/31083) and the Stab 4/5 and Stab 7/11 constructs were compared to untreated cells, scrambled siNA control constructs (Scram1 and Scram2), and cells transfected with lipid alone (transfection control). As shown in **Figure 23**, the original lead construct and the Stab 4/5 and Stab 7/11 modified siNA constructs all show significant reduction of BACE RNA expression.

Figure 24 shows a non-limiting example of the reduction of APP mRNA in SK-N-SH cells mediated by siNAs that target APP mRNA. SK-N-SH cells were transfected with 0.25 ug/well of lipid complexed with 25 nM siNA. Active chemically modified siNA constructs (solid bars; see **Tables III and IV**) were compared to untreated cells, matched chemistry irrelevant siNA control constructs (IC-1), and cells transfected with lipid alone (transfection control). As shown in **Figure 24**, the siNA constructs significantly reduce APP RNA expression compared with irrelevant siNA control and transfection control constructs.

Figure 25 shows a non-limiting example of the reduction of PSEN1 mRNA in SK-N-SH cells mediated by siNAs that target PSEN1 mRNA. SK-N-SH cells were transfected with 0.25 ug/well of lipid complexed with 25 nM siNA. Active chemically modified siNA constructs (solid bars; see **Tables III and IV**) were compared to untreated cells, matched chemistry irrelevant siNA control constructs (IC-1), and cells transfected with lipid alone (transfection control). As shown in **Figure 25**, the siNA constructs significantly reduce PSEN1 RNA expression compared with irrelevant siNA control and transfection control constructs.

Figure 26 shows a non-limiting example of the reduction of PSEN2 mRNA in SK-N-SH cells mediated by siNAs that target PSEN2 mRNA. SK-N-SH cells were transfected with 0.25 ug/well of lipid complexed with 25 nM siNA. Active chemically modified siNA constructs (solid bars; see **Tables III and IV**) were compared to untreated cells, matched chemistry irrelevant siNA control constructs (IC-1), and cells transfected with lipid alone (transfection control). As shown in **Figure 26**, the siNA

constructs significantly reduce PSEN2 RNA expression compared with irrelevant siNA control and transfection control constructs.

Example 10: Indications

Particular degenerative and disease states that can be associated with BACE, APP, PIN-1, PS-1 and/or PS-2 expression modulation include but are not limited to: Alzheimer's disease, dementia, stroke (CVA) and any other diseases or conditions that are related to the levels of BACE, APP, PIN-1, PS-1 and/or PS-2 in a cell or tissue, alone or in combination with other therapies. The reduction of BACE, APP, PIN-1, PS-1 and/or PS-2 expression (specifically BACE, APP, PIN-1, PS-1 and/or PS-2 RNA levels) and thus reduction in the level of the respective protein relieves, to some extent, the symptoms of the disease or condition.

Those skilled in the art will recognize that other drug compounds and therapies may be readily combined with or used in conjuction with the nucleic acid molecules of the instant invention (e.g., siNA molecules) are hence within the scope of the instant invention.

Example 11: Diagnostic uses

The siNA molecules of the invention can be used in a variety of diagnostic applications, such as in the identification of molecular targets (e.g., RNA) in a variety of applications, for example, in clinical, industrial, environmental, agricultural and/or research settings. Such diagnostic use of siNA molecules involves utilizing reconstituted RNAi systems, for example, using cellular lysates or partially purified cellular lysates. siNA molecules of this invention can be used as diagnostic tools to examine genetic drift and mutations within diseased cells or to detect the presence of endogenous or exogenous, for example viral, RNA in a cell. The close relationship between siNA activity and the structure of the target RNA allows the detection of mutations in any region of the molecule, which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple siNA molecules described in this invention, one can map nucleotide changes, which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with siNA molecules can be used to inhibit gene expression and define the role of specified gene products in the

progression of disease or infection. In this manner, other genetic targets can be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combination therapies (e.g., multiple siNA molecules targeted to different genes, siNA molecules coupled with known small molecule inhibitors, or intermittent treatment with combinations siNA molecules and/or other chemical or biological molecules). Other *in vitro* uses of siNA molecules of this invention are well known in the art, and include detection of the presence of mRNAs associated with a disease, infection, or related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a siNA using standard methodologies, for example, fluorescence resonance emission transfer (FRET).

In a specific example, siNA molecules that cleave only wild-type or mutant forms of the target RNA are used for the assay. The first siNA molecules (*i.e.*, those that cleave only wild-type forms of target RNA) are used to identify wild-type RNA present in the sample and the second siNA molecules (*i.e.*, those that cleave only mutant forms of target RNA) are used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA are cleaved by both siNA molecules to demonstrate the relative siNA efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus, each analysis requires two siNA molecules, two substrates and one unknown sample, which is combined into six reactions. The presence of cleavage products is determined using an RNase protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (*i.e.*, disease related or infection related) is adequate to establish risk. If probes of comparable specific activity are used for both transcripts, then a qualitative comparison of RNA levels is adequate and decreases the cost of the initial diagnosis. Higher mutant form to wild-type ratios are correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The methods and compositions described herein as presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art, which are encompassed within the spirit of the invention, are defined by the scope of the claims.

It will be readily apparent to one skilled in the art that varying substitutions and modifications can be made to the invention disclosed herein without departing from the scope and spirit of the invention. Thus, such additional embodiments are within the scope of the present invention and the following claims. The present invention teaches one skilled in the art to test various combinations and/or substitutions of chemical modifications described herein toward generating nucleic acid constructs with improved activity for mediating RNAi activity. Such improved activity can comprise improved stability, improved bioavailability, and/or improved activation of cellular responses mediating RNAi. Therefore, the specific embodiments described herein are not limiting and one skilled in the art can readily appreciate that specific combinations of the modifications described herein can be tested without undue experimentation toward identifying siNA molecules with improved RNAi activity.

The invention illustratively described herein suitably can be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible

within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the description and the appended claims.

In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

Table I: Accession Numbers

NM_012104
Homo sapiens beta-site APP-cleaving enzyme (BACE), transcript variant a, mRNA
gi|21040369|ref|NM_012104.2|[21040369]

NM_006222
Homo sapiens protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting 1-like (PIN1L), mRNA
gi|5453899|ref|NM_006222.1|[5453899]

L76517
Homo sapiens (clone cc44) senilin 1 (PS1; S182) mRNA, complete cds
gi|1479973|gb|L76517.1|HUMPS1MRNA[1479973]

L43964
Homo sapiens (clone F-T03796) STM-2 mRNA, complete cds
gi|951202|gb|L43964.1|HUMSTM2R[951202]

NM_138973
Homo sapiens beta-site APP-cleaving enzyme (BACE), transcript variant d, mRNA
gi|21040367|ref|NM_138973.1|[21040367]

NM_138972
Homo sapiens beta-site APP-cleaving enzyme (BACE), transcript variant b, mRNA
gi|21040365|ref|NM_138972.1|[21040365]

NM_138971
Homo sapiens beta-site APP-cleaving enzyme (BACE), transcript variant c, mRNA
gi|21040363|ref|NM_138971.1|[21040363]

AK075049

Homo sapiens cDNA FLJ90568 fis, clone OVARC1001570, highly similar to Homo
sapiens beta-site APP cleaving enzyme (BACE) mRNA
gi|22760888|dbj|AK075049.1|[22760888]

AF527782
Homo sapiens beta-site APP-cleaving enzyme (BACE) mRNA, partial cds,
alternatively spliced
gi|22094870|gb|AF527782.1|[22094870]

AF324837
Homo sapiens beta-site APP cleaving enzyme mRNA, partial cds, alternatively
spliced
gi|21449275|gb|AF324837.1|[21449275]

AF338817
Homo sapiens beta-site APP cleaving enzyme type C mRNA, complete cds
gi|13699247|gb|AF338817.1|[13699247]

AF338816
Homo sapiens beta-site APP cleaving enzyme type B mRNA, complete cds
gi|13699245|gb|AF338816.1|[13699245]

AB050438
Homo sapiens BACE mRNA for beta-site APP cleaving enzyme I-432, complete cds
gi|13568410|dbj|AB050438.1|[13568410]

AB050437
Homo sapiens BACE mRNA for beta-site APP cleaving enzyme I-457, complete cds
gi|13568408|dbj|AB050437.1|[13568408]

AB050436
Homo sapiens BACE mRNA for beta-site APP cleaving enzyme I-476, complete cds
gi|13568406|dbj|AB050436.1|[13568406]

AF190725
Homo sapiens beta-site APP cleaving enzyme (BACE) mRNA, complete cds
gi|6118538|gb|AF190725.1|AF190725[6118538]

NM_007319
Homo sapiens presenilin 1 (Alzheimer disease 3) (PSEN1), transcript variant I-374., mRNA
gi|7549814|ref|NM_007319.1|[7549814]

NM_138992
Homo sapiens beta-site APP-cleaving enzyme 2 (BACE2), transcript variant b, mRNA
gi|21040361|ref|NM_138992.1|[21040361]

NM_138991
Homo sapiens beta-site APP-cleaving enzyme 2 (BACE2), transcript variant c, mRNA
gi|21040359|ref|NM_138991.1|[21040359]

NM_012105
Homo sapiens beta-site APP-cleaving enzyme 2 (BACE2), transcript variant a, mRNA
gi|21040358|ref|NM_012105.3|[21040358]

AB066441
Homo sapiens APP mRNA for amyloid precursor protein, partial cds, D678N mutant
gi|16904654|dbj|AB066441.1|[16904654]

AB050438
Homo sapiens BACE mRNA for beta-site APP cleaving enzyme I-432, complete cds
gi|13568410|dbj|AB050438.1|[13568410]

AB050437
Homo sapiens BACE mRNA for beta-site APP cleaving enzyme I-457, complete cds
gi|13568408|dbj|AB050437.1|[13568408]

AB050436
Homo sapiens BACE mRNA for beta-site APP cleaving enzyme I-476, complete cds
gi|13568406|dbj|AB050436.1|[13568406]

NM_012486
Homo sapiens presenilin 2 (Alzheimer disease 4) (PSEN2), transcript variant 2, mRNA
gi|7108359|ref|NM_012486.1|[7108359]

NM_000447
Homo sapiens presenilin 2 (Alzheimer disease 4) (PSEN2), transcript variant 1, mRNA
gi|4506164|ref|NM_000447.1|[4506164]

AF188277
Homo sapiens aspartyl protease (BACE2) mRNA, complete cds, alternatively spliced
gi|7025334|gb|AF188277.1|AF188277[7025334]

AF188276
Homo sapiens aspartyl protease (BACE2) mRNA, complete cds, alternatively spliced
gi|7025332|gb|AF188276.1|AF188276[7025332]

AF178532
Homo sapiens aspartyl protease (BACE2) mRNA, complete cds
gi|6851265|gb|AF178532.1|AF178532[6851265]

D87675
Homo sapiens DNA for amyloid precursor protein, complete cds
gi|2429080|dbj|D87675.1|[2429080]

AF201468
Homo sapiens APP beta-secretase mRNA, complete cds
gi|6601444|gb|AF201468.1|AF201468[6601444]

AF190725
Homo sapiens beta-site APP cleaving enzyme (BACE) mRNA, complete cds
gi|6118538|gb|AF190725.1|AF190725[6118538]

E14707
DNA encoding a mutated amyloid precursor protein
gi|5709390|dbj|E14707.1||pat|JP|1998001499|1[5709390]

AF168956
Homo sapiens amyloid precursor protein homolog HSD-2 mRNA, complete cds
gi|5702387|gb|AF168956.1|AF168956[5702387]

S60099
APPH=amyloid precursor protein homolog [human, placenta, mRNA, 3727 nt]
gi|300168|bbm|300685|bbs|131198|gb|S60099.1|S60099[300168]

U50939
Human amyloid precursor protein-binding protein 1 mRNA, complete cds
gi|1314559|gb|U50939.1|HSU50939[1314559]

NM_000484
Homo sapiens amyloid beta (A4) precursor protein (protease nexin-II, Alzheimer disease) (APP), transcript variant 1, mRNA
gi|41406053|ref|NM_000484.2|[41406053]

BC018937
Homo sapiens amyloid beta (A4) precursor protein (protease nexin-II, Alzheimer disease), mRNA (cDNA clone IMAGE:4126584)
gi|39645179|gb|BC018937.2|[39645179]

NM_201413
Homo sapiens amyloid beta (A4) precursor protein (protease nexin-II, Alzheimer

disease) (APP), transcript variant 2, mRNA
gi|41406054|ref|NM_201413.1|[41406054]

NM_201414
Homo sapiens amyloid beta (A4) precursor protein (protease nexin-II, Alzheimer
disease) (APP), transcript variant 3, mRNA
gi|41406056|ref|NM_201414.1|[41406056]

BC065529
Homo sapiens amyloid beta (A4) precursor protein (protease nexin-II, Alzheimer
disease), transcript variant 2, mRNA (cDNA clone MGC:75167 IMAGE:6152423),
complete cds
gi|41350938|gb|BC065529.1|[41350938]

Y00264
Human mRNA for amyloid A4 precursor of Alzheimer's disease
gi|28525|emb|Y00264.1|HSAFPA4[28525]

AF282245
Homo sapiens amyloid precursor protein 639 (APP639) mRNA, complete cds
gi|33339673|gb|AF282245.1|[33339673]

X06989
Homo sapiens mRNA for amyloid A4 protein (APP gene)
gi|28720|emb|X06989.1|HSAPA4R[28720]

TABLE II: APP, BACE, PSEN1, PSEN2 siNA AND TARGET SEQUENCES

APP NM_000484

Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
3	UUUCCUCCGGCAGCGGUAGG	1	3	UUUCCUCCGGCAGCGGUAGG	1	21	CCUACCGCUGCCGAGGAAA	200
21	GCGAGAGCACGCGGAGGAG	2	21	GCGAGAGCACGCGGAGGAG	2	39	CUCCUCCGCGUGCUCUCGC	201
39	GCGUGCGCGGGGGCCCCGG	3	39	GCGUGCGCGGGGGCCCCGG	3	57	CCGGGGCCCCCGCGCACGC	202
57	GGAGACGGCGCGGCGGUGGCG	4	57	GGAGACGGCGCGGCGGUGGCG	4	75	CGCCACCGCCGCCGUCUCC	203
75	GGCGCGGGCAGAGCAAGGA	5	75	GGCGCGGGCAGAGCAAGGA	5	93	UCCUUGCUCUGCCCCGCGCC	204
93	ACGCGGCGGAUCCCACUCG	6	93	ACGCGGCGGAUCCCACUCG	6	111	CGAGUGGGAUCCGCCGCGU	205
111	GCACAGCAGCGCACUCGGU	7	111	GCACAGCAGCGCACUCGGU	7	129	ACCGAGUGCGCUGCUGUGC	206
129	UGCCCCGCGCAGGGUCGCG	8	129	UGCCCCGCGCAGGGUCGCG	8	147	CGCGACCCUGCGCGGGGCA	207
147	GAUGCUGCCCGGUUUGGCA	9	147	GAUGCUGCCCGGUUUGGCA	9	165	UGCCAAACCGGGCAGCAUC	208
165	ACUGCUCCUGCUGGCCGCC	10	165	ACUGCUCCUGCUGGCCGCC	10	183	GGCGGCCAGCAGGAGCAGU	209
183	CUGGACGGCUCGGGCGCUG	11	183	CUGGACGGCUCGGGCGCUG	11	201	CAGCGCCCGAGCCGUCCAG	210
201	GGAGGUACCCACUGAUGGU	12	201	GGAGGUACCCACUGAUGGU	12	219	ACCAUCAGUGGGUACCUCC	211
219	UAAUGCUGGCCUGCUGGCU	13	219	UAAUGCUGGCCUGCUGGCU	13	237	AGCCAGCAGGCCAGCAUUA	212
237	UGAACCCCAGAUUGCCAUG	14	237	UGAACCCCAGAUUGCCAUG	14	255	CAUGGCAAUCUGGGGUUCA	213
255	GUUCUGUGGCAGACUGAAC	15	255	GUUCUGUGGCAGACUGAAC	15	273	GUUCAGUCUGCCACAGAAC	214
273	CAUGCACAUGAAUGUCCAG	16	273	CAUGCACAUGAAUGUCCAG	16	291	CUGGACAUUCAUGUGCAUG	215
291	GAAUGGGAAGUGGGAUUCA	17	291	GAAUGGGAAGUGGGAUUCA	17	309	UGAAUCCCACUUCCCAUUC	216
309	AGAUCCAUCAGGGACCAAA	18	309	AGAUCCAUCAGGGACCAAA	18	327	UUUGGUCCCUGAUGGAUCU	217
327	AACCUGCAUUGAUACCAAG	19	327	AACCUGCAUUGAUACCAAG	19	345	CUUGGUAUCAAUGCAGGUU	218
345	GGAAGGCAUCCUGCAGUAU	20	345	GGAAGGCAUCCUGCAGUAU	20	363	AUACUGCAGGAUGCCUUCC	219
363	UUGCCAAGAAGUCUACCCU	21	363	UUGCCAAGAAGUCUACCCU	21	381	AGGGUAGACUUCUUGGCAA	220
381	UGAACUGCAGAUCACCAAU	22	381	UGAACUGCAGAUCACCAAU	22	399	AUUGGUGAUCUGCAGUUCA	221
399	UGUGGUAGAAGCCAACCAA	23	399	UGUGGUAGAAGCCAACCAA	23	417	UUGGUUGGCUUCUACCACA	222
417	ACCAGUGACCAUCCAGAAC	24	417	ACCAGUGACCAUCCAGAAC	24	435	GUUCUGGAUGGUCACUGGU	223
435	CUGGUGCAAGCGGGGCCGC	25	435	CUGGUGCAAGCGGGGCCGC	25	453	GCGGCCCCGCUUGCACCAG	224
453	CAAGCAGUGCAAGACCCAU	26	453	CAAGCAGUGCAAGACCCAU	26	471	AUGGGUCUUGCACUGCUUG	225
471	UCCCCACUUUGUGAUUCCC	27	471	UCCCCACUUUGUGAUUCCC	27	489	GGGAAUCACAAAGUGGGGA	226
489	CUACCGCUGCUUAGUUGGU	28	489	CUACCGCUGCUUAGUUGGU	28	507	ACCAACUAAGCAGCGGUAG	227
507	UGAGUUUGUAAGUGAUGCC	29	507	UGAGUUUGUAAGUGAUGCC	29	525	GGCAUCACUUACAAACUCA	228

525	CCUUCUCGUUCCUGACAAG	30	525	CCUUCUCGUUCCUGACAAG	30	543	CUUGUCAGGAACGAGAAGG	229
543	GUGCAAAUUCUUACACCAG	31	543	GUGCAAAUUCUUACACCAG	31	561	CUGGUGUAAGAAUUUGCAC	230
561	GGAGAGGAUGGAUGUUUGC	32	561	GGAGAGGAUGGAUGUUUGC	32	579	GCAAACAUCCAUCCUCUCC	231
579	CGAAACUCAUCUUCACUGG	33	579	CGAAACUCAUCUUCACUGG	33	597	CCAGUGAAGAUGAGUUUCG	232
597	GCACACCGUCGCCAAAGAG	34	597	GCACACCGUCGCCAAAGAG	34	615	CUCUUUGGCGACGGUGUGC	233
615	GACAUGCAGUGAGAAGAGU	35	615	GACAUGCAGUGAGAAGAGU	35	633	ACUCUUCUCACUGCAUGUC	234
633	UACCAACUUGCAUGACUAC	36	633	UACCAACUUGCAUGACUAC	36	651	GUAGUCAUGCAAGUUGGUA	235
651	CGGCAUGUUGCUGCCCUGC	37	651	CGGCAUGUUGCUGCCCUGC	37	669	GCAGGGCAGCAACAUGCCG	236
669	CGGAAUUGACAAGUUCCGA	38	669	CGGAAUUGACAAGUUCCGA	38	687	UCGGAACUUGUCAAUUCCG	237
687	AGGGGUAGAGUUUGUGUGU	39	687	AGGGGUAGAGUUUGUGUGU	39	705	ACACACAAACUCUACCCCU	238
705	UUGCCCACUGGCUGAAGAA	40	705	UUGCCCACUGGCUGAAGAA	40	723	UUCUUCAGCCAGUGGGCAA	239
723	AAGUGACAAUGUGGAUUCU	41	723	AAGUGACAAUGUGGAUUCU	41	741	AGAAUCCACAUUGUCACUU	240
741	UGCUGAUGCGGAGGAGGAU	42	741	UGCUGAUGCGGAGGAGGAU	42	759	AUCCUCCUCCGCAUCAGCA	241
759	UGACUCGGAUGUCUGGUGG	43	759	UGACUCGGAUGUCUGGUGG	43	777	CCACCAGACAUCCGAGUCA	242
777	GGGCGGAGCAGACACAGAC	44	777	GGGCGGAGCAGACACAGAC	44	795	GUCUGUGUCUGCUCCGCCC	243
795	CUAUGCAGAUGGGAGUGAA	45	795	CUAUGCAGAUGGGAGUGAA	45	813	UUCACUCCCAUCUGCAUAG	244
813	AGACAAAGUAGUAGAAGUA	46	813	AGACAAAGUAGUAGAAGUA	46	831	UACUUCUACUACUUUGUCU	245
831	AGCAGAGGAGGAAGAAGUG	47	831	AGCAGAGGAGGAAGAAGUG	47	849	CACUUCUUCCUCCUCUGCU	246
849	GGCUGAGGUGGAAGAAGAA	48	849	GGCUGAGGUGGAAGAAGAA	48	867	UUCUUCUUCCACCUCAGCC	247
867	AGAAGCCGAUGAUGACGAG	49	867	AGAAGCCGAUGAUGACGAG	49	885	CUCGUCAUCAUCGGCUUCU	248
885	GGACGAUGAGGAUGGUGAU	50	885	GGACGAUGAGGAUGGUGAU	50	903	AUCACCAUCCUCAUCGUCC	249
903	UGAGGUAGAGGAAGAGGCU	51	903	UGAGGUAGAGGAAGAGGCU	51	921	AGCCUCUUCCUCUACCUCA	250
921	UGAGGAACCCUACGAAGAA	52	921	UGAGGAACCCUACGAAGAA	52	939	UUCUUCGUAGGGUUCCUCA	251
939	AGCCACAGAGAGAACCACC	53	939	AGCCACAGAGAGAACCACC	53	957	GGUGGUUCUCUCUGUGGCU	252
957	CAGCAUUGCCACCACCACC	54	957	CAGCAUUGCCACCACCACC	54	975	GGUGGUGGUGGCAAUGCUG	253
975	CACCACCACCACAGAGUCU	55	975	CACCACCACCACAGAGUCU	55	993	AGACUCUGUGGUGGUGGUG	254
993	UGUGGAAGAGGUGGUUCGA	56	993	UGUGGAAGAGGUGGUUCGA	56	1011	UCGAACCACCUCUUCCACA	255
1011	AGAGGUGUGCUCUGAACAA	57	1011	AGAGGUGUGCUCUGAACAA	57	1029	UUGUUCAGAGCACACCUCU	256
1029	AGCCGAGACGGGGCCGUGC	58	1029	AGCCGAGACGGGGCCGUGC	58	1047	GCACGGCCCCGUCUCGGCU	257
1047	CCGAGCAAUGAUCUCCCGC	59	1047	CCGAGCAAUGAUCUCCCGC	59	1065	GCGGGAGAUCAUUGCUCGG	258
1065	CUGGUACUUUGAUGUGACU	60	1065	CUGGUACUUUGAUGUGACU	60	1083	AGUCACAUCAAAGUACCAG	259
1083	UGAAGGGAAGUGUGCCCCA	61	1083	UGAAGGGAAGUGUGCCCCA	61	1101	UGGGGCACACUUCCCUUCA	260
1101	AUUCUUUUACGGCGGAUGU	62	1101	AUUCUUUUACGGCGGAUGU	62	1119	ACAUCCGCCGUAAAAGAAU	261
1119	UGGCGGCAACCGGAACAAC	63	1119	UGGCGGCAACCGGAACAAC	63	1137	GUUGUUCCGGUUGCCGCCA	262

1137	CUUUGACACAGAAGAGUAC	64	1137	CUUUGACACAGAAGAGUAC	64	1155	GUACUCUUCUGUGUCAAAG	263
1155	CUGCAUGGCCGUGUGUGGC	65	1155	CUGCAUGGCCGUGUGUGGC	65	1173	GCCACACACGGCCAUGCAG	264
1173	CAGCGCCAUGUCCCAAAGU	66	1173	CAGCGCCAUGUCCCAAAGU	66	1191	ACUUUGGGACAUGGCGCUG	265
1191	UUUACUCAAGACUACCCAG	67	1191	UUUACUCAAGACUACCCAG	67	1209	CUGGGUAGUCUUGAGUAAA	266
1209	GGAACCUCUUGCCCGAGAU	68	1209	GGAACCUCUUGCCCGAGAU	68	1227	AUCUCGGGCAAGAGGUUCC	267
1227	UCCUGUUAAACUUCCUACA	69	1227	UCCUGUUAAACUUCCUACA	69	1245	UGUAGGAAGUUUAACAGGA	268
1245	AACAGCAGCCAGUACCCCU	70	1245	AACAGCAGCCAGUACCCCU	70	1263	AGGGGUACUGGCUGCUGUU	269
1263	UGAUGCCGUUGACAAGUAU	71	1263	UGAUGCCGUUGACAAGUAU	71	1281	AUACUUGUCAACGGCAUCA	270
1281	UCUCGAGACACCUGGGGAU	72	1281	UCUCGAGACACCUGGGGAU	72	1299	AUCCCCAGGUGUCUCGAGA	271
1299	UGAGAAUGAACAUGCCCAU	73	1299	UGAGAAUGAACAUGCCCAU	73	1317	AUGGGCAUGUUCAUUCUCA	272
1317	UUUCCAGAAAGCCAAAGAG	74	1317	UUUCCAGAAAGCCAAAGAG	74	1335	CUCUUUGGCUUUCUGGAAA	273
1335	GAGGCUUGAGGCCAAGCAC	75	1335	GAGGCUUGAGGCCAAGCAC	75	1353	GUGCUUGGCCUCAAGCCUC	274
1353	CCGAGAGAGAAUGUCCCAG	76	1353	CCGAGAGAGAAUGUCCCAG	76	1371	CUGGGACAUUCUCUCUCGG	275
1371	GGUCAUGAGAGAAUGGGAA	77	1371	GGUCAUGAGAGAAUGGGAA	77	1389	UUCCCAUUCUCUCAUGACC	276
1389	AGAGGCAGAACGUCAAGCA	78	1389	AGAGGCAGAACGUCAAGCA	78	1407	UGCUUGACGUUCUGCCUCU	277
1407	AAAGAACUUGCCUAAAGCU	79	1407	AAAGAACUUGCCUAAAGCU	79	1425	AGCUUUAGGCAAGUUCUUU	278
1425	UGAUAAGAAGGCAGUUAUC	80	1425	UGAUAAGAAGGCAGUUAUC	80	1443	GAUAACUGCCUUCUUAUCA	279
1443	CCAGCAUUUCCAGGAGAAA	81	1443	CCAGCAUUUCCAGGAGAAA	81	1461	UUUCUCCUGGAAAUGCUGG	280
1461	AGUGGAAUCUUUGGAACAG	82	1461	AGUGGAAUCUUUGGAACAG	82	1479	CUGUUCCAAAGAUUCCACU	281
1479	GGAAGCAGCCAACGAGAGA	83	1479	GGAAGCAGCCAACGAGAGA	83	1497	UCUCUCGUUGGCUGCUUCC	282
1497	ACAGCAGCUGGUGGAGACA	84	1497	ACAGCAGCUGGUGGAGACA	84	1515	UGUCUCCACCAGCUGCUGU	283
1515	ACACAUGGCCAGAGUGGAA	85	1515	ACACAUGGCCAGAGUGGAA	85	1533	UUCCACUCUGGCCAUGUGU	284
1533	AGCCAUGCUCAAUGACCGC	86	1533	AGCCAUGCUCAAUGACCGC	86	1551	GCGGUCAUUGAGCAUGGCU	285
1551	CCGCCGCCUGGCCCUGGAG	87	1551	CCGCCGCCUGGCCCUGGAG	87	1569	CUCCAGGGCCAGGCGGCGG	286
1569	GAACUACAUCACCGCUCUG	88	1569	GAACUACAUCACCGCUCUG	88	1587	CAGAGCGGUGAUGUAGUUC	287
1587	GCAGGCUGUUCCUCCUCGG	89	1587	GCAGGCUGUUCCUCCUCGG	89	1605	CCGAGGAGGAACAGCCUGC	288
1605	GCCUCGUCACGUGUUCAAU	90	1605	GCCUCGUCACGUGUUCAAU	90	1623	AUUGAACACGUGACGAGGC	289
1623	UAUGCUAAAGAAGUAUGUC	91	1623	UAUGCUAAAGAAGUAUGUC	91	1641	GACAUACUUCUUUAGCAUA	290
1641	CCGCGCAGAACAGAAGGAC	92	1641	CCGCGCAGAACAGAAGGAC	92	1659	GUCCUUCUGUUCUGCGCGG	291
1659	CAGACAGCACACCCUAAAG	93	1659	CAGACAGCACACCCUAAAG	93	1677	CUUUAGGGUGUGCUGUCUG	292
1677	GCAUUUCGAGCAUGUGCGC	94	1677	GCAUUUCGAGCAUGUGCGC	94	1695	GCGCACAUGCUCGAAAUGC	293
1695	CAUGGUGGAUCCCAAGAAA	95	1695	CAUGGUGGAUCCCAAGAAA	95	1713	UUUCUUGGGAUCCACCAUG	294
1713	AGCCGCUCAGAUCCGGUCC	96	1713	AGCCGCUCAGAUCCGGUCC	96	1731	GGACCGGAUCUGAGCGGCU	295
1731	CCAGGUUAUGACACACCUC	97	1731	CCAGGUUAUGACACACCUC	97	1749	GAGGUGUGUCAUAACCUGG	296

1749	CCGUGUGAUUUAUGAGCGC	98	1749	CCGUGUGAUUUAUGAGCGC	98	1767	GCGCUCAUAAAUCACACGG	297
1767	CAUGAAUCAGUCUCUCUCC	99	1767	CAUGAAUCAGUCUCUCUCC	99	1785	GGAGAGAGACUGAUUCAUG	298
1785	CCUGCUCUACAACGUGCCU	100	1785	CCUGCUCUACAACGUGCCU	100	1803	AGGCACGUUGUAGAGCAGG	299
1803	UGCAGUGGCCGAGGAGAUU	101	1803	UGCAGUGGCCGAGGAGAUU	101	1821	AAUCUCCUCGGCCACUGCA	300
1821	UCAGGAUGAAGUUGAUGAG	102	1821	UCAGGAUGAAGUUGAUGAG	102	1839	CUCAUCAACUUCAUCCUGA	301
1839	GCUGCUUCAGAAAGAGCAA	103	1839	GCUGCUUCAGAAAGAGCAA	103	1857	UUGCUCUUUCUGAAGCAGC	302
1857	AAACUAUUCAGAUGACGUC	104	1857	AAACUAUUCAGAUGACGUC	104	1875	GACGUCAUCUGAAUAGUUU	303
1875	CUUGGCCAACAUGAUUAGU	105	1875	CUUGGCCAACAUGAUUAGU	105	1893	ACUAAUCAUGUUGGCCAAG	304
1893	UGAACCAAGGAUCAGUUAC	106	1893	UGAACCAAGGAUCAGUUAC	106	1911	GUAACUGAUCCUUGGUUCA	305
1911	CGGAAACGAUGCUCUCAUG	107	1911	CGGAAACGAUGCUCUCAUG	107	1929	CAUGAGAGCAUCGUUUCCG	306
1929	GCCAUCUUUGACCGAAACG	108	1929	GCCAUCUUUGACCGAAACG	108	1947	CGUUUCGGUCAAAGAUGGC	307
1947	GAAAACCACCGUGGAGCUC	109	1947	GAAAACCACCGUGGAGCUC	109	1965	GAGCUCCACGGUGGUUUUC	308
1965	CCUUCCCGUGAAUGGAGAG	110	1965	CCUUCCCGUGAAUGGAGAG	110	1983	CUCUCCAUUCACGGGAAGG	309
1983	GUUCAGCCUGGACGAUCUC	111	1983	GUUCAGCCUGGACGAUCUC	111	2001	GAGAUCGUCCAGGCUGAAC	310
2001	CCAGCCGUGGCAUUCUUUU	112	2001	CCAGCCGUGGCAUUCUUUU	112	2019	AAAAGAAUGCCACGGCUGG	311
2019	UGGGGCUGACUCUGUGCCA	113	2019	UGGGGCUGACUCUGUGCCA	113	2037	UGGCACAGAGUCAGCCCCA	312
2037	AGCCAACACAGAAAACGAA	114	2037	AGCCAACACAGAAAACGAA	114	2055	UUCGUUUUCUGUGUUGGCU	313
2055	AGUUGAGCCUGUUGAUGCC	115	2055	AGUUGAGCCUGUUGAUGCC	115	2073	GGCAUCAACAGGCUCAACU	314
2073	CCGCCCUGCUGCCGACCGA	116	2073	CCGCCCUGCUGCCGACCGA	116	2091	UCGGUCGGCAGCAGGGCGG	315
2091	AGGACUGACCACUCGACCA	117	2091	AGGACUGACCACUCGACCA	117	2109	UGGUCGAGUGGUCAGUCCU	316
2109	AGGUUCUGGGUUGACAAAU	118	2109	AGGUUCUGGGUUGACAAAU	118	2127	AUUUGUCAACCCAGAACCU	317
2127	UAUCAAGACGGAGGAGAUC	119	2127	UAUCAAGACGGAGGAGAUC	119	2145	GAUCUCCUCCGUCUUGAUA	318
2145	CUCUGAAGUGAAGAUGGAU	120	2145	CUCUGAAGUGAAGAUGGAU	120	2163	AUCCAUCUUCACUUCAGAG	319
2163	UGCAGAAUUCCGACAUGAC	121	2163	UGCAGAAUUCCGACAUGAC	121	2181	GUCAUGUCGGAAUUCUGCA	320
2181	CUCAGGAUAUGAAGUUCAU	122	2181	CUCAGGAUAUGAAGUUCAU	122	2199	AUGAACUUCAUAUCCUGAG	321
2199	UCAUCAAAAAUUGGUGUUC	123	2199	UCAUCAAAAAUUGGUGUUC	123	2217	GAACACCAAUUUUUGAUGA	322
2217	CUUUGCAGAAGAUGUGGGU	124	2217	CUUUGCAGAAGAUGUGGGU	124	2235	ACCCACAUCUUCUGCAAAG	323
2235	UUCAAACAAAGGUGCAAUC	125	2235	UUCAAACAAAGGUGCAAUC	125	2253	GAUUGCACCUUUGUUUGAA	324
2253	CAUUGGACUCAUGGUGGGC	126	2253	CAUUGGACUCAUGGUGGGC	126	2271	GCCCACCAUGAGUCCAAUG	325
2271	CGGUGUUGUCAUAGCGACA	127	2271	CGGUGUUGUCAUAGCGACA	127	2289	UGUCGCUAUGACAACACCG	326
2289	AGUGAUCGUCAUCACCUUG	128	2289	AGUGAUCGUCAUCACCUUG	128	2307	CAAGGUGAUGACGAUCACU	327
2307	GGUGAUGCUGAAGAAGAAA	129	2307	GGUGAUGCUGAAGAAGAAA	129	2325	UUUCUUCUUCAGCAUCACC	328
2325	ACAGUACACAUCCAUUCAU	130	2325	ACAGUACACAUCCAUUCAU	130	2343	AUGAAUGGAUGUGUACUGU	329
2343	UCAUGGUGUGGUGGAGGUU	131	2343	UCAUGGUGUGGUGGAGGUU	131	2361	AACCUCCACCACACCAUGA	330

2361	UGACGCCGCUGUCACCCCA	132	2361	UGACGCCGCUGUCACCCCA	132	2379	UGGGGUGACAGCGGCGUCA	331
2379	AGAGGAGCGCCACCUGUCC	133	2379	AGAGGAGCGCCACCUGUCC	133	2397	GGACAGGUGGCGCUCCUCU	332
2397	CAAGAUGCAGCAGAACGGC	134	2397	CAAGAUGCAGCAGAACGGC	134	2415	GCCGUUCUGCUGCAUCUUG	333
2415	CUACGAAAAUCCAACCUAC	135	2415	CUACGAAAAUCCAACCUAC	135	2433	GUAGGUUGGAUUUUCGUAG	334
2433	CAAGUUCUUUGAGCAGAUG	136	2433	CAAGUUCUUUGAGCAGAUG	136	2451	CAUCUGCUCAAAGAACUUG	335
2451	GCAGAACUAGACCCCCGCC	137	2451	GCAGAACUAGACCCCCGCC	137	2469	GGCGGGGGUCUAGUUCUGC	336
2469	CACAGCAGCCUCUGAAGUU	138	2469	CACAGCAGCCUCUGAAGUU	138	2487	AACUUCAGAGGCUGCUGUG	337
2487	UGGACAGCAAAACCAUUGC	139	2487	UGGACAGCAAAACCAUUGC	139	2505	GCAAUGGUUUUGCUGUCCA	338
2505	CUUCACUACCCAUCGGUGU	140	2505	CUUCACUACCCAUCGGUGU	140	2523	ACACCGAUGGGUAGUGAAG	339
2523	UCCAUUUAUAGAAUAAUGU	141	2523	UCCAUUUAUAGAAUAAUGU	141	2541	ACAUUAUUCUAUAAAUGGA	340
2541	UGGGAAGAAACAAACCCGU	142	2541	UGGGAAGAAACAAACCCGU	142	2559	ACGGGUUUGUUUCUUCCCA	341
2559	UUUUAUGAUUUACUCAUUA	143	2559	UUUUAUGAUUUACUCAUUA	143	2577	UAAUGAGUAAAUCAUAAAA	342
2577	AUCGCCUUUUGACAGCUGU	144	2577	AUCGCCUUUUGACAGCUGU	144	2595	ACAGCUGUCAAAAGGCGAU	343
2595	UGCUGUAACACAAGUAGAU	145	2595	UGCUGUAACACAAGUAGAU	145	2613	AUCUACUUGUGUUACAGCA	344
2613	UGCCUGAACUUGAAUUAAU	146	2613	UGCCUGAACUUGAAUUAAU	146	2631	AUUAAUUCAAGUUCAGGCA	345
2631	UCCACACAUCAGUAAUGUA	147	2631	UCCACACAUCAGUAAUGUA	147	2649	UACAUUACUGAUGUGUGGA	346
2649	AUUCUAUCUCUCUUUACAU	148	2649	AUUCUAUCUCUCUUUACAU	148	2667	AUGUAAAGAGAGAUAGAAU	347
2667	UUUUGGUCUCUAUACUACA	149	2667	UUUUGGUCUCUAUACUACA	149	2685	UGUAGUAUAGAGACCAAAA	348
2685	AUUAUUAAUGGGUUUUGUG	150	2685	AUUAUUAAUGGGUUUUGUG	150	2703	CACAAAACCCAUUAAUAAU	349
2703	GUACUGUAAAGAAUUUAGC	151	2703	GUACUGUAAAGAAUUUAGC	151	2721	GCUAAAUUCUUUACAGUAC	350
2721	CUGUAUCAAACUAGUGCAU	152	2721	CUGUAUCAAACUAGUGCAU	152	2739	AUGCACUAGUUUGAUACAG	351
2739	UGAAUAGAUUCUCUCCUGA	153	2739	UGAAUAGAUUCUCUCCUGA	153	2757	UCAGGAGAGAAUCUAUUCA	352
2757	AUUAUUUAUCACAUAGCCC	154	2757	AUUAUUUAUCACAUAGCCC	154	2775	GGGCUAUGUGAUAAAUAAU	353
2775	CCUUAGCCAGUUGUAUAUU	155	2775	CCUUAGCCAGUUGUAUAUU	155	2793	AAUAUACAACUGGCUAAGG	354
2793	UAUUCUUGUGGUUUGUGAC	156	2793	UAUUCUUGUGGUUUGUGAC	156	2811	GUCACAAACCACAAGAAUA	355
2811	CCCAAUUAAGUCCUACUUU	157	2811	CCCAAUUAAGUCCUACUUU	157	2829	AAAGUAGGACUUAAUUGGG	356
2829	UACAUAUGCUUUAAGAAUC	158	2829	UACAUAUGCUUUAAGAAUC	158	2847	GAUUCUUAAAGCAUAUGUA	357
2847	CGAUGGGGGAUGCUUCAUG	159	2847	CGAUGGGGGAUGCUUCAUG	159	2865	CAUGAAGCAUCCCCCAUCG	358
2865	GUGAACGUGGGAGUUCAGC	160	2865	GUGAACGUGGGAGUUCAGC	160	2883	GCUGAACUCCCCACGUUCAC	359
2883	CUGCUUCUCUUGCCUAAGU	161	2883	CUGCUUCUCUUGCCUAAGU	161	2901	ACUUAGGCAAGAGAAGGCAG	360
2901	UAUUCCUUUCCUGAUCACU	162	2901	UAUUCCUUUCCUGAUCACU	162	2919	AGUGAUCAGGAAAGGAAUA	361
2919	UAUGCAUUUUAAAGUUAAA	163	2919	UAUGCAUUUUAAAGUUAAA	163	2937	UUUAACUUUAAAAUGCAUA	362
2937	ACAUUUUUAAGUAUUUCAG	164	2937	ACAUUUUUAAGUAUUUCAG	164	2955	CUGAAAUACUUAAAAAUGU	363
2955	GAUGCUUUAGAGAGAUUUU	165	2955	GAUGCUUUAGAGAGAUUUU	165	2973	AAAAUCUCUCUAAAGCAUC	364

2973	UUUUUCCAUGACUGCAUUU	166	2973	UUUUUCCAUGACUGCAUUU	166	2991	AAAUGCAGUCAUGGAAAAA	365
2991	UUACUGUACAGAUUGCUGC	167	2991	UUACUGUACAGAUUGCUGC	167	3009	GCAGCAAUCUGUACAGUAA	366
3009	CUUCUGCUAUAUUUGUGAU	168	3009	CUUCUGCUAUAUUUGUGAU	168	3027	AUCACAAAUAUAGCAGAAG	367
3027	UAUAGGAAUUAAGAGGAUA	169	3027	UAUAGGAAUUAAGAGGAUA	169	3045	UAUCCUCUUAAUUCCUAUA	368
3045	ACACACGUUUGUUUCUUCG	170	3045	ACACACGUUUGUUUCUUCG	170	3063	CGAAGAAACAAACGUGUGU	369
3063	GUGCCUGUUUUAUGUGCAC	171	3063	GUGCCUGUUUUAUGUGCAC	171	3081	GUGCACAUAAAACAGGCAC	370
3081	CACAUUAGGCAUUGAGACU	172	3081	CACAUUAGGCAUUGAGACU	172	3099	AGUCUCAAUGCCUAAUGUG	371
3099	UUCAAGCUUUUCUUUUUUU	173	3099	UUCAAGCUUUUCUUUUUUU	173	3117	AAAAAAAGAAAAGCUUGAA	372
3117	UGUCCACGUAUCUUUGGGU	174	3117	UGUCCACGUAUCUUUGGGU	174	3135	ACCCAAAGAUACGUGGACA	373
3135	UCUUUGAUAAAGAAAAGAA	175	3135	UCUUUGAUAAAGAAAAGAA	175	3153	UUCUUUUCUUUAUCAAAGA	374
3153	AUCCCUGUUCAUUGUAAGC	176	3153	AUCCCUGUUCAUUGUAAGC	176	3171	GCUUACAAUGAACAGGGAU	375
3171	CACUUUUACGGGGCGGGUG	177	3171	CACUUUUACGGGGCGGGUG	177	3189	CACCCGCCCCGUAAAAGUG	376
3189	GGGGAGGGGUGCUCUGCUG	178	3189	GGGGAGGGGUGCUCUGCUG	178	3207	CAGCAGAGCACCCCUCCCC	377
3207	GGUCUUCAAUUACCAAGAA	179	3207	GGUCUUCAAUUACCAAGAA	179	3225	UUCUUGGUAAUUGAAGACC	378
3225	AUUCUCCAAAACAAUUUUC	180	3225	AUUCUCCAAAACAAUUUUC	180	3243	GAAAAUUGUUUUGGAGAAU	379
3243	CUGCAGGAUGAUUGUACAG	181	3243	CUGCAGGAUGAUUGUACAG	181	3261	CUGUACAAUCAUCCUGCAG	380
3261	GAAUCAUUGCUUAUGACAU	182	3261	GAAUCAUUGCUUAUGACAU	182	3279	AUGUCAUAAGCAAUGAUUC	381
3279	UGAUCGCUUUCUACACUGU	183	3279	UGAUCGCUUUCUACACUGU	183	3297	ACAGUGUAGAAAGCGAUCA	382
3297	UAUUACAUAAAUAAAUUAA	184	3297	UAUUACAUAAAUAAAUUAA	184	3315	UUAAUUUAUUUAUGUAAUA	383
3315	AAUAAAAUAACCCCGGGCA	185	3315	AAUAAAAUAACCCCGGGCA	185	3333	UGCCCGGGGUUAUUUUAUU	384
3333	AAGACUUUUCUUUGAAGGA	186	3333	AAGACUUUUCUUUGAAGGA	186	3351	UCCUUCAAAGAAAAGUCUU	385
3351	AUGACUACAGACAUUAAAU	187	3351	AUGACUACAGACAUUAAAU	187	3369	AUUUAAUGUCUGUAGUCAU	386
3369	UAAUCGAAGUAAUUUUUGGG	188	3369	UAAUCGAAGUAAUUUUUGGG	188	3387	CCCAAAAUUACUUCGAUUA	387
3387	GUGGGGAGAAGAGGCAGAU	189	3387	GUGGGGAGAAGAGGCAGAU	189	3405	AUCUGCCUCUUCUCCCCAC	388
3405	UUCAAUUUUCUUUAACCAG	190	3405	UUCAAUUUUCUUUAACCAG	190	3423	CUGGUUAAAGAAAAUUGAA	389
3423	GUCUGAAGUUUCAUUUAUG	191	3423	GUCUGAAGUUUCAUUUAUG	191	3441	CAUAAAUGAAACUUCAGAC	390
3441	GAUACAAAAGAAGAUGAAA	192	3441	GAUACAAAAGAAGAUGAAA	192	3459	UUUCAUCUUCUUUUGUAUC	391
3459	AAUGGAAGUGGCAAUAUAA	193	3459	AAUGGAAGUGGCAAUAUAA	193	3477	UUAUAUUGCCACUUCCAUU	392
3477	AGGGGAUGAGGAAGGCAUG	194	3477	AGGGGAUGAGGAAGGCAUG	194	3495	CAUGCCUUCCUCAUCCCCU	393
3495	GCCUGGACAAACCCUUCUU	195	3495	GCCUGGACAAACCCUUCUU	195	3513	AAGAAGGGUUUGUCCAGGC	394
3513	UUUAAGAUGUGUCUUCAAU	196	3513	UUUAAGAUGUGUCUUCAAU	196	3531	AUUGAAGACACAUCUUAAA	395
3531	UUUGUAUAAAAUGGUGUUU	197	3531	UUUGUAUAAAAUGGUGUUU	197	3549	AAACACCAUUUUAUACAAA	396
3549	UUCAUGUAAAUAAAUACAU	198	3549	UUCAUGUAAAUAAAUACAU	198	3567	AUGUAUUUAUUUACAUGAA	397
3559	UAAAUACAUUCUUGGAGGA	199	3559	UAAAUACAUUCUUGGAGGA	199	3577	UCCUCCAAGAAUGUAUUUA	398

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Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
1	CGCACUCGUCCCCAGCCCG	399	1	CGCACUCGUCCCCAGCCCG	399	19	CGGGCUGGGGACGAGUGCG	724
19	GCCCGGGAGCUGCGAGCCG	400	19	GCCCGGGAGCUGCGAGCCG	400	37	CGGCUCGCAGCUCCCGGGC	725
37	GCGAGCUGGAUUAUGGUGG	401	37	GCGAGCUGGAUUAUGGUGG	401	55	CCACCAUAAUCCAGCUCGC	726
55	GCCUGAGCAGCCAACGCAG	402	55	GCCUGAGCAGCCAACGCAG	402	73	CUGCGUUGGCUGCUCAGGC	727
73	GCCGCAGGAGCCCGGAGCC	403	73	GCCGCAGGAGCCCGGAGCC	403	91	GGCUCCGGGCUCCUGCGGC	728
91	CCUUGCCCCUGCCCGCGCC	404	91	CCUUGCCCCUGCCCGCGCC	404	109	GGCGCGGGCAGGGGCAAGG	729
109	CGCCGCCCGCCGGGGGGAC	405	109	CGCCGCCCGCCGGGGGGAC	405	127	GUCCCCCCGGCGGGCGGCG	730
127	CCAGGGAAGCCGCCACCGG	406	127	CCAGGGAAGCCGCCACCGG	406	145	CCGGUGGCGGCUUCCCUGG	731
145	GCCCGCCAUGCCCGCCCCU	407	145	GCCCGCCAUGCCCGCCCCU	407	163	AGGGGCGGGCAUGGCGGGC	732
163	UCCCAGCCCCGCCGGGAGC	408	163	UCCCAGCCCCGCCGGGAGC	408	181	GCUCCCGGCGGGGCUGGGA	733
181	CCCGCGCCCGCUGCCCAGG	409	181	CCCGCGCCCGCUGCCCAGG	409	199	CCUGGGCAGCGGGCGCGGG	734
199	GCUGGCCGCCGCCGUGCCG	410	199	GCUGGCCGCCGCCGUGCCG	410	217	CGGCACGGCGGCGGCCAGC	735
217	GAUGUAGCGGGCUCCGGAU	411	217	GAUGUAGCGGGCUCCGGAU	411	235	AUCCGGAGCCCGCUACAUC	736
235	UCCCAGCCUCUCCCCUGCU	412	235	UCCCAGCCUCUCCCCUGCU	412	253	AGCAGGGGAGAGGCUGGGA	737
253	UCCCGUGCUCUGCGGAUCU	413	253	UCCCGUGCUCUGCGGAUCU	413	271	AGAUCCGCAGAGCACGGGA	738
271	UCCCCUGACCGCUCUCCAC	414	271	UCCCCUGACCGCUCUCCAC	414	289	GUGGAGAGCGGUCAGGGGA	739
289	CAGCCCGGACCCGGGGGCU	415	289	CAGCCCGGACCCGGGGGCU	415	307	AGCCCCCGGGUCCGGGCUG	740
307	UGGCCCAGGGCCCUGCAGG	416	307	UGGCCCAGGGCCCUGCAGG	416	325	CCUGCAGGGCCCUGGGCCA	741
325	GCCCUGGCGUCCUGAUGCC	417	325	GCCCUGGCGUCCUGAUGCC	417	343	GGCAUCAGGACGCCAGGGC	742
343	CCCCAAGCUCCCUCUCCUG	418	343	CCCCAAGCUCCCUCUCCUG	418	361	CAGGAGAGGGAGCUUGGGG	743
361	GAGAAGCCACCAGCACCAC	419	361	GAGAAGCCACCAGCACCAC	419	379	GUGGUGCUGGUGGCUUCUC	744
379	CCCAGACUUGGGGGCAGGC	420	379	CCCAGACUUGGGGGCAGGC	420	397	GCCUGCCCCCAAGUCUGGG	745
397	CGCCAGGGACGGACGUGGG	421	397	CGCCAGGGACGGACGUGGG	421	415	CCCACGUCCGUCCCUGGCG	746
415	GCCAGUGCGAGCCCAGAGG	422	415	GCCAGUGCGAGCCCAGAGG	422	433	CCUCUGGGCUCGCACUGGC	747
433	GGCCCGAAGGCCGGGGCCC	423	433	GGCCCGAAGGCCGGGGCCC	423	451	GGGCCCCGGCCUUCGGGCC	748
451	CACCAUGGCCCAAGCCCUG	424	451	CACCAUGGCCCAAGCCCUG	424	469	CAGGGCUUGGGCCAUGGUG	749
469	GCCCUGGCUCCUGCUGUGG	425	469	GCCCUGGCUCCUGCUGUGG	425	487	CCACAGCAGGAGCCAGGGC	750
487	GAUGGGCGCGGGAGUGCUG	426	487	GAUGGGCGCGGGAGUGCUG	426	505	CAGCACUCCCGCGCCCAUC	751
505	GCCUGCCCACGGCACCCAG	427	505	GCCUGCCCACGGCACCCAG	427	523	CUGGGUGCCGUGGGCAGGC	752

523	GCACGGCAUCCGGCUGCCC	428	523	GCACGGCAUCCGGCUGCCC	428	541	GGGCAGCCGGAUGCCGUGC	753
541	CCUGCGCAGCGGCCUGGGG	429	541	CCUGCGCAGCGGCCUGGGG	429	559	CCCCAGGCCGCUGCGCAGG	754
559	GGGCGCCCCCCUGGGGCUG	430	559	GGGCGCCCCCCUGGGGCUG	430	577	CAGCCCCAGGGGGGCGCCC	755
577	GCGGCUGCCCCCGGGAGACC	431	577	GCGGCUGCCCCCGGGAGACC	431	595	GGUCUCCCGGGGCAGCCGC	756
595	CGACGAAGAGCCCGAGGAG	432	595	CGACGAAGAGCCCGAGGAG	432	613	CUCCUCGGGCUCUUCGUCG	757
613	GCCCGGCCGGAGGGGCAGC	433	613	GCCCGGCCGGAGGGGCAGC	433	631	GCUGCCCCUCCGGCCGGGC	758
631	CUUUGUGGAGAUGGUGGAC	434	631	CUUUGUGGAGAUGGUGGAC	434	649	GUCCACCAUCUCCACAAAG	759
649	CAACCUGAGGGGCAAGUCG	435	649	CAACCUGAGGGGCAAGUCG	435	667	CGACUUGCCCCUCAGGUUG	760
667	GGGGCAGGGCUACUACGUG	436	667	GGGGCAGGGCUACUACGUG	436	685	CACGUAGUAGCCCUGCCCC	761
685	GGAGAUGACCGUGGGCAGC	437	685	GGAGAUGACCGUGGGCAGC	437	703	GCUGCCCACGGUCAUCUCC	762
703	CCCCCCGCAGACGCUCAAC	438	703	CCCCCCGCAGACGCUCAAC	438	721	GUUGAGCGUCUGCGGGGGG	763
721	CAUCCUGGUGGAUACAGGC	439	721	CAUCCUGGUGGAUACAGGC	439	739	GCCUGUAUCCACCAGGAUG	764
739	CAGCAGUAACUUUGCAGUG	440	739	CAGCAGUAACUUUGCAGUG	440	757	CACUGCAAAGUUACUGCUG	765
757	GGGUGCUGCCCCCCACCCC	441	757	GGGUGCUGCCCCCCACCCC	441	775	GGGGUGGGGGGCAGCACCC	766
775	CUUCCUGCAUCGCUACUAC	442	775	CUUCCUGCAUCGCUACUAC	442	793	GUAGUAGCGAUGCAGGAAG	767
793	CCAGAGGCAGCUGUCCAGC	443	793	CCAGAGGCAGCUGUCCAGC	443	811	GCUGGACAGCUGCCUCUGG	768
811	CACAUACCGGGACCUCCGG	444	811	CACAUACCGGGACCUCCGG	444	829	CCGGAGGUCCCCGGUAUGUG	769
829	GAAGGGUGUGUAUGUGCCC	445	829	GAAGGGUGUGUAUGUGCCC	445	847	GGGCACAUACACACCCUUC	770
847	CUACACCCAGGGCAAGUGG	446	847	CUACACCCAGGGCAAGUGG	446	865	CCACUUGCCCUGGGUGUAG	771
865	GGAAGGGGAGCUGGGCACC	447	865	GGAAGGGGAGCUGGGCACC	447	883	GGUGCCCAGCUCCCCUUCC	772
883	CGACCUGGUAAGCAUCCCC	448	883	CGACCUGGUAAGCAUCCCC	448	901	GGGGAUGCUUACCAGGUCG	773
901	CCAUGGCCCCAACGUCACU	449	901	CCAUGGCCCCAACGUCACU	449	919	AGUGACGUUGGGGCCAUGG	774
919	UGUGCGUGCCAACAUUGCU	450	919	UGUGCGUGCCAACAUUGCU	450	937	AGCAAUGUUGGCACGCACA	775
937	UGCCAUCACUGAAUCAGAC	451	937	UGCCAUCACUGAAUCAGAC	451	955	GUCUGAUUCAGUGAUGGCA	776
955	CAAGUUCUUCAUCAACGGC	452	955	CAAGUUCUUCAUCAACGGC	452	973	GCCGUUGAUGAAGAACUUG	777
973	CUCCAACUGGGAAGGCAUC	453	973	CUCCAACUGGGAAGGCAUC	453	991	GAUGCCUUCCCAGUUGGAG	778
991	CCUGGGGCUGGCCUAUGCU	454	991	CCUGGGGCUGGCCUAUGCU	454	1009	AGCAUAGGCCAGCCCCAGG	779
1009	UGAGAUUGCCAGGCCUGAC	455	1009	UGAGAUUGCCAGGCCUGAC	455	1027	GUCAGGCCUGGCAAUCUCA	780
1027	CGACUCCCUGGAGCCUUUC	456	1027	CGACUCCCUGGAGCCUUUC	456	1045	GAAAGGCUCCAGGGAGUCG	781
1045	CUUUGACUCUCUGGUAAAG	457	1045	CUUUGACUCUCUGGUAAAG	457	1063	CUUUACCAGAGAGUCAAAG	782
1063	GCAGACCCACGUUCCCAAC	458	1063	GCAGACCCACGUUCCCAAC	458	1081	GUUGGGAACGUGGGUCUGC	783
1081	CCUCUUCUCCCUGCAGCUU	459	1081	CCUCUUCUCCCUGCAGCUU	459	1099	AAGCUGCAGGGAGAAGAGG	784
1099	UUGUGGUGCUGGCUUCCCC	460	1099	UUGUGGUGCUGGCUUCCCC	460	1117	GGGGAAGCCAGCACCACAA	785
1117	CCUCAACCAGUCUGAAGUG	461	1117	CCUCAACCAGUCUGAAGUG	461	1135	CACUUCAGACUGGUUGAGG	786

1135	GCUGGCCUCUGUCGGAGGG	462	1135	GCUGGCCUCUGUCGGAGGG	462	1153	CCCUCCGACAGAGGCCAGC	787
1153	GAGCAUGAUCAUUGGAGGU	463	1153	GAGCAUGAUCAUUGGAGGU	463	1171	ACCUCCAAUGAUCAUGCUC	788
1171	UAUCGACCACUCGCUGUAC	464	1171	UAUCGACCACUCGCUGUAC	464	1189	GUACAGCGAGUGGUCGAUA	789
1189	CACAGGCAGUCUCUGGUAU	465	1189	CACAGGCAGUCUCUGGUAU	465	1207	AUACCAGAGACUGCCUGUG	790
1207	UACACCCAUCCGGCGGGAG	466	1207	UACACCCAUCCGGCGGGAG	466	1225	CUCCCGCCGGAUGGGUGUA	791
1225	GUGGUAUUAUGAGGUCAUC	467	1225	GUGGUAUUAUGAGGUCAUC	467	1243	GAUGACCUCAUAAUACCAC	792
1243	CAUUGUGCGGGUGGAGAUC	468	1243	CAUUGUGCGGGUGGAGAUC	468	1261	GAUCUCCACCCGCACAAUG	793
1261	CAAUGGACAGGAUCUGAAA	469	1261	CAAUGGACAGGAUCUGAAA	469	1279	UUUCAGAUCCUGUCCAUUG	794
1279	AAUGGACUGCAAGGAGUAC	470	1279	AAUGGACUGCAAGGAGUAC	470	1297	GUACUCCUUGCAGUCCAUU	795
1297	CAACUAUGACAAGAGCAUU	471	1297	CAACUAUGACAAGAGCAUU	471	1315	AAUGCUCUUGUCAUAGUUG	796
1315	UGUGGACAGUGGCACCACC	472	1315	UGUGGACAGUGGCACCACC	472	1333	GGUGGUGCCACUGUCCACA	797
1333	CAACCUUCGUUUGCCCAAG	473	1333	CAACCUUCGUUUGCCCAAG	473	1351	CUUGGGCAAACGAAGGUUG	798
1351	GAAAGUGUUUGAAGCUGCA	474	1351	GAAAGUGUUUGAAGCUGCA	474	1369	UGCAGCUUCAAACACUUUC	799
1369	AGUCAAAUCCAUCAAGGCA	475	1369	AGUCAAAUCCAUCAAGGCA	475	1387	UGCCUUGAUGGAUUUGACU	800
1387	AGCCUCCUCCACGGAGAAG	476	1387	AGCCUCCUCCACGGAGAAG	476	1405	CUUCUCCGUGGAGGAGGCU	801
1405	GUUCCCUGAUGGUUUCUGG	477	1405	GUUCCCUGAUGGUUUCUGG	477	1423	CCAGAAACCAUCAGGGAAC	802
1423	GCUAGGAGAGCAGCUGGUG	478	1423	GCUAGGAGAGCAGCUGGUG	478	1441	CACCAGCUGCUCUCCUAGC	803
1441	GUGCUGGCAAGCAGGCACC	479	1441	GUGCUGGCAAGCAGGCACC	479	1459	GGUGCCUGCUUGCCAGCAC	804
1459	CACCCCUUGGAACAUUUUC	480	1459	CACCCCUUGGAACAUUUUC	480	1477	GAAAAUGUUCCAAGGGUG	805
1477	CCCAGUCAUCUCACUCUAC	481	1477	CCCAGUCAUCUCACUCUAC	481	1495	GUAGAGUGAGAUGACUGGG	806
1495	CCUAAUGGGUGAGGUUACC	482	1495	CCUAAUGGGUGAGGUUACC	482	1513	GGUAACCUCACCCAUUAGG	807
1513	CAACCAGUCCUUCCGCAUC	483	1513	CAACCAGUCCUUCCGCAUC	483	1531	GAUGCGGAAGGACUGGUUG	808
1531	CACCAUCCUUCCGCAGCAA	484	1531	CACCAUCCUUCCGCAGCAA	484	1549	UUGCUGCGGAAGGAUGGUG	809
1549	AUACCUGCGGCCAGUGGAA	485	1549	AUACCUGCGGCCAGUGGAA	485	1567	UUCCACUGGCCGCAGGUAU	810
1567	AGAUGUGGCCACGUCCCAA	486	1567	AGAUGUGGCCACGUCCCAA	486	1585	UUGGGACGUGGCCACAUCU	811
1585	AGACGACUGUUACAAGUUU	487	1585	AGACGACUGUUACAAGUUU	487	1603	AAACUUGUAACAGUCGUCU	812
1603	UGCCAUCUCACAGUCAUCC	488	1603	UGCCAUCUCACAGUCAUCC	488	1621	GGAUGACUGUGAGAUGGCA	813
1621	CACGGGCACUGUUAUGGGA	489	1621	CACGGGCACUGUUAUGGGA	489	1639	UCCCAUAACAGUGCCCGUG	814
1639	AGCUGUUAUCAUGGAGGGC	490	1639	AGCUGUUAUCAUGGAGGGC	490	1657	GCCCUCCAUGAUAACAGCU	815
1657	CUUCUACGUUGUCUUUGAU	491	1657	CUUCUACGUUGUCUUUGAU	491	1675	AUCAAAGACAACGUAGAAG	816
1675	UCGGGCCCGAAAACGAAUU	492	1675	UCGGGCCCGAAAACGAAUU	492	1693	AAUUCGUUUUCGGGCCCGA	817
1693	UGGCUUUGCUGUCAGCGCU	493	1693	UGGCUUUGCUGUCAGCGCU	493	1711	AGCGCUGACAGCAAAGCCA	818
1711	UUGCCAUGUGCACGAUGAG	494	1711	UUGCCAUGUGCACGAUGAG	494	1729	CUCAUCGUGCACAUGGCAA	819
1729	GUUCAGGACGGCAGCGGUG	495	1729	GUUCAGGACGGCAGCGGUG	495	1747	CACCGCUGCCGUCCUGAAC	820

1747	GGAAGGCCCUUUUGUCACC	496	1747	GGAAGGCCCUUUUGUCACC	496	1765	GGUGACAAAAGGGCCUUCC	821
1765	CUUGGACAUGGAAGACUGU	497	1765	CUUGGACAUGGAAGACUGU	497	1783	ACAGUCUUCCAUGUCCAAG	822
1783	UGGCUACAACAUUCCACAG	498	1783	UGGCUACAACAUUCCACAG	498	1801	CUGUGGAAUGUUGUAGCCA	823
1801	GACAGAUGAGUCAACCCUC	499	1801	GACAGAUGAGUCAACCCUC	499	1819	GAGGGUUGACUCAUCUGUC	824
1819	CAUGACCAUAGCCUAUGUC	500	1819	CAUGACCAUAGCCUAUGUC	500	1837	GACAUAGGCUAUGGUCAUG	825
1837	CAUGGCUGCCAUCUGCGCC	501	1837	CAUGGCUGCCAUCUGCGCC	501	1855	GGCGCAGAUGGCAGCCAUG	826
1855	CCUCUUCAUGCUGCCACUC	502	1855	CCUCUUCAUGCUGCCACUC	502	1873	GAGUGGCAGCAUGAAGAGG	827
1873	CUGCCUCAUGGUGUGUCAG	503	1873	CUGCCUCAUGGUGUGUCAG	503	1891	CUGACACACCAUGAGGCAG	828
1891	GUGGCGCUGCCUCCGCUGC	504	1891	GUGGCGCUGCCUCCGCUGC	504	1909	GCAGCGGAGGCAGCGCCAC	829
1909	CCUGCGCCAGCAGCAUGAU	505	1909	CCUGCGCCAGCAGCAUGAU	505	1927	AUCAUGCUGCUGGCGCAGG	830
1927	UGACUUUGCUGAUGACAUC	506	1927	UGACUUUGCUGAUGACAUC	506	1945	GAUGUCAUCAGCAAAGUCA	831
1945	CUCCCUGCUGAAGUGAGGA	507	1945	CUCCCUGCUGAAGUGAGGA	507	1963	UCCUCACUUCAGCAGGGAG	832
1963	AGGCCCAUGGGCAGAAGAU	508	1963	AGGCCCAUGGGCAGAAGAU	508	1981	AUCUUCUGCCCAUGGGCCU	833
1981	UAGAGAUUCCCCUGGACCA	509	1981	UAGAGAUUCCCCUGGACCA	509	1999	UGGUCCAGGGGAAUCUCUA	834
1999	ACACCUCCGUGGUUCACUU	510	1999	ACACCUCCGUGGUUCACUU	510	2017	AAGUGAACCACGGAGGUGU	835
2017	UUGGUCACAAGUAGGAGAC	511	2017	UUGGUCACAAGUAGGAGAC	511	2035	GUCUCCUACUUGUGACCAA	836
2035	CACAGAUGGCACCUGUGGC	512	2035	CACAGAUGGCACCUGUGGC	512	2053	GCCACAGGUGCCAUCUGUG	837
2053	CCAGAGCACCUCAGGACCC	513	2053	CCAGAGCACCUCAGGACCC	513	2071	GGGUCCUGAGGUGCUCUGG	838
2071	CUCCCCACCCACCAAAUGC	514	2071	CUCCCCACCCACCAAAUGC	514	2089	GCAUUUGGUGGGUGGGGAG	839
2089	CCUCUGCCUUGAUGGAGAA	515	2089	CCUCUGCCUUGAUGGAGAA	515	2107	UUCUCCAUCAAGGCAGAGG	840
2107	AGGAAAAGGCUGGCAAGGU	516	2107	AGGAAAAGGCUGGCAAGGU	516	2125	ACCUUGCCAGCCUUUUCCU	841
2125	UGGGUUCCAGGGACUGUAC	517	2125	UGGGUUCCAGGGACUGUAC	517	2143	GUACAGUCCCUGGAACCCA	842
2143	CCUGUAGGAAACAGAAAAG	518	2143	CCUGUAGGAAACAGAAAAG	518	2161	CUUUUCUGUUUCCUACAGG	843
2161	GAGAAGAAAGAAGCACUCU	519	2161	GAGAAGAAAGAAGCACUCU	519	2179	AGAGUGCUUCUUUCUUCUC	844
2179	UGCUGGCGGGAAUACUCUU	520	2179	UGCUGGCGGGAAUACUCUU	520	2197	AAGAGUAUUCCCGCCAGCA	845
2197	UGGUCACCUCAAAUUUAAG	521	2197	UGGUCACCUCAAAUUUAAG	521	2215	CUUAAAUUUGAGGUGACCA	846
2215	GUCGGGAAAUUCUGCUGCU	522	2215	GUCGGGAAAUUCUGCUGCU	522	2233	AGCAGCAGAAUUUCCCGAC	847
2233	UUGAAACUUCAGCCCUGAA	523	2233	UUGAAACUUCAGCCCUGAA	523	2251	UUCAGGGCUGAAGUUUCAA	848
2251	ACCUUUGUCCACCAUUCCU	524	2251	ACCUUUGUCCACCAUUCCU	524	2269	AGGAAUGGUGGACAAAGGU	849
2269	UUUAAAUUCUCCAACCCAA	525	2269	UUUAAAUUCUCCAACCCAA	525	2287	UUGGGUUGGAGAAUUUAAA	850
2287	AAGUAUUCUUCUUUUCUUA	526	2287	AAGUAUUCUUCUUUUCUUA	526	2305	UAAGAAAAGAAGAAUACUU	851
2305	AGUUUCAGAAGUACUGGCA	527	2305	AGUUUCAGAAGUACUGGCA	527	2323	UGCCAGUACUUCUGAAACU	852
2323	AUCACACGCAGGUUACCUU	528	2323	AUCACACGCAGGUUACCUU	528	2341	AAGGUAACCUGCGGUGUGAU	853
2341	UGGCGUGUGUCCCUGUGGU	529	2341	UGGCGUGUGUCCCUGUGGU	529	2359	ACCACAGGGACACACGCCA	854

2359	UACCCUGGCAGAGAAGAGA	530	2359	UACCCUGGCAGAGAAGAGA	530	2377	UCUCUUCUCUGCCAGGGUA	855
2377	ACCAAGCUUGUUUCCCUGC	531	2377	ACCAAGCUUGUUUCCCUGC	531	2395	GCAGGGAAACAAGCUUGGU	856
2395	CUGGCCAAAGUCAGUAGGA	532	2395	CUGGCCAAAGUCAGUAGGA	532	2413	UCCUACUGACUUUGGCCAG	857
2413	AGAGGAUGCACAGUUUGCU	533	2413	AGAGGAUGCACAGUUUGCU	533	2431	AGCAAACUGUGCAUCCUCU	858
2431	UAUUUGCUUUAGAGACAGG	534	2431	UAUUUGCUUUAGAGACAGG	534	2449	CCUGUCUCUAAAGCAAAUA	859
2449	GGACUGUAUAAACAAGCCU	535	2449	GGACUGUAUAAACAAGCCU	535	2467	AGGCUUGUUUAUACAGUCC	860
2467	UAACAUUGGUGCAAAGAUU	536	2467	UAACAUUGGUGCAAAGAUU	536	2485	AAUCUUUGCACCAAUGUUA	861
2485	UGCCUCUUGAAUUAAAAAA	537	2485	UGCCUCUUGAAUUAAAAAA	537	2503	UUUUUUAAUUCAAGAGGCA	862
2503	AAAAAACUAGAUUGACUAU	538	2503	AAAAAACUAGAUUGACUAU	538	2521	AUAGUCAAUCUAGUUUUUU	863
2521	UUUAUACAAAUGGGGGCGG	539	2521	UUUAUACAAAUGGGGGCGG	539	2539	CCGCCCCCAUUUGUAUAAA	864
2539	GCUGGAAAGAGGAGAAGGA	540	2539	GCUGGAAAGAGGAGAAGGA	540	2557	UCCUUCUCCUCUUUCCAGC	865
2557	AGAGGGAGUACAAAGACAG	541	2557	AGAGGGAGUACAAAGACAG	541	2575	CUGUCUUUGUACUCCCUCU	866
2575	GGGAAUAGUGGGAUCAAAG	542	2575	GGGAAUAGUGGGAUCAAAG	542	2593	CUUUGAUCCCACUAUUCCC	867
2593	GCUAGGAAAGGCAGAAACA	543	2593	GCUAGGAAAGGCAGAAACA	543	2611	UGUUUCUGCCUUUCCUAGC	868
2611	ACAACCACUCACCAGUCCU	544	2611	ACAACCACUCACCAGUCCU	544	2629	AGGACUGGUGAGUGGUUGU	869
2629	UAGUUUUAGACCUCAUCUC	545	2629	UAGUUUUAGACCUCAUCUC	545	2647	GAGAUGAGGUCUAAAACUA	870
2647	CCAAGAUAGCAUCCCAUCU	546	2647	CCAAGAUAGCAUCCCAUCU	546	2665	AGAUGGGAUGCUAUCUUGG	871
2665	UCAGAAGAUGGGUGUUGUU	547	2665	UCAGAAGAUGGGUGUUGUU	547	2683	AACAACACCCAUCUUCUGA	872
2683	UUUCAAUGUUUUCUUUUCU	548	2683	UUUCAAUGUUUUCUUUUCU	548	2701	AGAAAAGAAAACAUUGAAA	873
2701	UGUGGUUGCAGCCUGACCA	549	2701	UGUGGUUGCAGCCUGACCA	549	2719	UGGUCAGGCUGCAACCACA	874
2719	AAAAGUGAGAUGGGAAGGG	550	2719	AAAAGUGAGAUGGGAAGGG	550	2737	CCCUUCCCAUCUCACUUUU	875
2737	GCUUAUCUAGCCAAAGAGC	551	2737	GCUUAUCUAGCCAAAGAGC	551	2755	GCUCUUUGGCUAGAUAAGC	876
2755	CUCUUUUUAGCUCUCUUA	552	2755	CUCUUUUUAGCUCUCUUA	552	2773	UAAGAGAGCUAAAAAAGAG	877
2773	AAAUGAAGUGCCCACUAAG	553	2773	AAAUGAAGUGCCCACUAAG	553	2791	CUUAGUGGGCACUUCAUUU	878
2791	GAAGUUCCACUUAACACAU	554	2791	GAAGUUCCACUUAACACAU	554	2809	AUGUGUUAAGUGGAACUUC	879
2809	UGAAUUUCUGCCAUAUUAA	555	2809	UGAAUUUCUGCCAUAUUAA	555	2827	UUAAUAUGGCAGAAAUUCA	880
2827	AUUUCAUUGUCUCUAUCUG	556	2827	AUUUCAUUGUCUCUAUCUG	556	2845	CAGAUAGAGACAAUGAAAU	881
2845	GAACCACCCUUUAUUCUAC	557	2845	GAACCACCCUUUAUUCUAC	557	2863	GUAGAAUAAAGGGUGGUUC	882
2863	CAUAUGAUAGGCAGCACUG	558	2863	CAUAUGAUAGGCAGCACUG	558	2881	CAGUGCUGCCUAUCAUAUG	883
2881	GAAAUAUCCUAACCCCCUA	559	2881	GAAAUAUCCUAACCCCCUA	559	2899	UAGGGGGUUAGGAUAUUUC	884
2899	AAGCUCCAGGUGCCCUGUG	560	2899	AAGCUCCAGGUGCCCUGUG	560	2917	CACAGGGCACCUGGAGCUU	885
2917	GGGAGAGCAACUGGACUAU	561	2917	GGGAGAGCAACUGGACUAU	561	2935	AUAGUCCAGUUGCUCUCCC	886
2935	UAGCAGGGCUGGGCUCUGU	562	2935	UAGCAGGGCUGGGCUCUGU	562	2953	ACAGAGCCCAGCCCUGCUA	887
2953	UCUUCCUGGUCAUAGGCUC	563	2953	UCUUCCUGGUCAUAGGCUC	563	2971	GAGCCUAUGACCAGGAAGA	888

2971	CACUCUUUCCCCCAAAUCU	564	2971	CACUCUUUCCCCCAAAUCU	564	2989	AGAUUUGGGGGAAAGAGUG	889
2989	UUCCUCUGGAGCUUUGCAG	565	2989	UUCCUCUGGAGCUUUGCAG	565	3007	CUGCAAAGCUCCAGAGGAA	890
3007	GCCAAGGUGCUAAAAGGAA	566	3007	GCCAAGGUGCUAAAAGGAA	566	3025	UUCCUUUUAGCACCUUGGC	891
3025	AUAGGUAGGAGACCUCUUC	567	3025	AUAGGUAGGAGACCUCUUC	567	3043	GAAGAGGUCUCCUACCUAU	892
3043	CUAUCUAAUCCUUAAAAGC	568	3043	CUAUCUAAUCCUUAAAAGC	568	3061	GCUUUUAAGGAUUAGAUAG	893
3061	CAUAAUGUUGAACAUUCAU	569	3061	CAUAAUGUUGAACAUUCAU	569	3079	AUGAAUGUUCAACAUUAUG	894
3079	UUCAACAGCUGAUGCCCUA	570	3079	UUCAACAGCUGAUGCCCUA	570	3097	UAGGGCAUCAGCUGUUGAA	895
3097	AUAACCCCUGCCUGGAUUU	571	3097	AUAACCCCUGCCUGGAUUU	571	3115	AAAUCCAGGCAGGGGUUAU	896
3115	UCUUCCUAUUAGGCUAUAA	572	3115	UCUUCCUAUUAGGCUAUAA	572	3133	UUAUAGCCUAAUAGGAAGA	897
3133	AGAAGUAGCAAGAUCUUUA	573	3133	AGAAGUAGCAAGAUCUUUA	573	3151	UAAAGAUCUUGCUACUUCU	898
3151	ACAUAAUUCAGAGUGGUUU	574	3151	ACAUAAUUCAGAGUGGUUU	574	3169	AAACCACUCUGAAUUAUGU	899
3169	UCAUUGCCUUCCUACCCUC	575	3169	UCAUUGCCUUCCUACCCUC	575	3187	GAGGGUAGGAAGGCAAUGA	900
3187	CUCUAAUGGCCCCUCCAUU	576	3187	CUCUAAUGGCCCCUCCAUU	576	3205	AAUGGAGGGGCCAUUAGAG	901
3205	UUAUUUGACUAAAGCAUCA	577	3205	UUAUUUGACUAAAGCAUCA	577	3223	UGAUGCUUUAGUCAAAUAA	902
3223	ACACAGUGGCACUAGCAUU	578	3223	ACACAGUGGCACUAGCAUU	578	3241	AAUGCUAGUGCCACUGUGU	903
3241	UAUACCAAGAGUAUGAGAA	579	3241	UAUACCAAGAGUAUGAGAA	579	3259	UUCUCAUACUCUUGGUAUA	904
3259	AAUACAGUGCUUUAUGGCU	580	3259	AAUACAGUGCUUUAUGGCU	580	3277	AGCCAUAAAGCACUGUAUU	905
3277	UCUAACAUUACUGCCUUCA	581	3277	UCUAACAUUACUGCCUUCA	581	3295	UGAAGGCAGUAAUGUUAGA	906
3295	AGUAUCAAGGCUGCCUGGA	582	3295	AGUAUCAAGGCUGCCUGGA	582	3313	UCCAGGCAGCCUUGAUACU	907
3313	AGAAAGGAUGGCAGCCUCA	583	3313	AGAAAGGAUGGCAGCCUCA	583	3331	UGAGGCUGCCAUCCUUUCU	908
3331	AGGGCUUCCUUAUGUCCUC	584	3331	AGGGCUUCCUUAUGUCCUC	584	3349	GAGGACAUAAGGAAGCCCU	909
3349	CCACCACAAGAGCUCCUUG	585	3349	CCACCACAAGAGCUCCUUG	585	3367	CAAGGAGCUCUUGUGGUGG	910
3367	GAUGAAGGUCAUCUUUUUC	586	3367	GAUGAAGGUCAUCUUUUUC	586	3385	GAAAAAGAUGACCUUCAUC	911
3385	CCCCUAUCCUGUUCUUCCC	587	3385	CCCCUAUCCUGUUCUUCCC	587	3403	GGGAAGAACAGGAUAGGGG	912
3403	CCUCCCCGCUCCUAAUGGU	588	3403	CCUCCCCGCUCCUAAUGGU	588	3421	ACCAUUAGGAGCGGGGAGG	913
3421	UACGUGGGUACCCAGGCUG	589	3421	UACGUGGGUACCCAGGCUG	589	3439	CAGCCUGGGUACCCACGUA	914
3439	GGUUCUUGGGCUAGGUAGU	590	3439	GGUUCUUGGGCUAGGUAGU	590	3457	ACUACCUAGCCCAAGAACC	915
3457	UGGGGACCAAGUUCAUUAC	591	3457	UGGGGACCAAGUUCAUUAC	591	3475	GUAAUGAACUUGGUCCCCA	916
3475	CCUCCCUAUCAGUUCUAGC	592	3475	CCUCCCUAUCAGUUCUAGC	592	3493	GCUAGAACUGAUAGGGAGG	917
3493	CAUAGUAAACUACGGUACC	593	3493	CAUAGUAAACUACGGUACC	593	3511	GGUACCGUAGUUUACUAUG	918
3511	CAGUGUUAGUGGGAAGAGC	594	3511	CAGUGUUAGUGGGAAGAGC	594	3529	GCUCUUCCCACUAACACUG	919
3529	CUGGGUUUUCCUAGUAUAC	595	3529	CUGGGUUUUCCUAGUAUAC	595	3547	GUAUACUAGGAAAACCCAG	920
3547	CCCACUGCAUCCUACUCCU	596	3547	CCCACUGCAUCCUACUCCU	596	3565	AGGAGUAGGAUGCAGUGGG	921
3565	UACCUGGUCAACCCGCUGC	597	3565	UACCUGGUCAACCCGCUGC	597	3583	GCAGCGGGUUGACCAGGUA	922

3583	CUUCCAGGUAUGGGACCUG	598	3583	CUUCCAGGUAUGGGACCUG	598	3601	CAGGUCCCAUACCUGGAAG	923
3601	GCUAAGUGUGGAAUUACCU	599	3601	GCUAAGUGUGGAAUUACCU	599	3619	AGGUAAUUCCACACUUAGC	924
3619	UGAUAAGGGAGAGGGAAAU	600	3619	UGAUAAGGGAGAGGGAAAU	600	3637	AUUUCCCUCUCCCCUUAUCA	925
3637	UACAAGGAGGGCCUCUGGU	601	3637	UACAAGGAGGGCCUCUGGU	601	3655	ACCAGAGGCCCUCCUUGUA	926
3655	UGUUCCUGGCCUCAGCCAG	602	3655	UGUUCCUGGCCUCAGCCAG	602	3673	CUGGCUGAGGCCAGGAACA	927
3673	GCUGCCCACAAGCCAUAAA	603	3673	GCUGCCCACAAGCCAUAAA	603	3691	UUUAUGGCUUGUGGGCAGC	928
3691	ACCAAUAAAACAAGAAUAC	604	3691	ACCAAUAAAACAAGAAUAC	604	3709	GUAUUCUUGUUUUAUUGGU	929
3709	CUGAGUCAGUUUUUUAUCU	605	3709	CUGAGUCAGUUUUUUAUCU	605	3727	AGAUAAAAAACUGACUCAG	930
3727	UGGGUUCUCUUCAUUCCCA	606	3727	UGGGUUCUCUUCAUUCCCA	606	3745	UGGGAAUGAAGAGAACCCA	931
3745	ACUGCACUUGGUGCUGCUU	607	3745	ACUGCACUUGGUGCUGCUU	607	3763	AAGCAGCACCAAGUGCAGU	932
3763	UUGGCUGACUGGGAACACC	608	3763	UUGGCUGACUGGGAACACC	608	3781	GGUGUUCCCAGUCAGCCAA	933
3781	CCCAUAACUACAGAGUCUG	609	3781	CCCAUAACUACAGAGUCUG	609	3799	CAGACUCUGUAGUUAUGGG	934
3799	GACAGGAAGACUGGAGACU	610	3799	GACAGGAAGACUGGAGACU	610	3817	AGUCUCCAGUCUUCCUGUC	935
3817	UGUCCACUUCUAGCUCGGA	611	3817	UGUCCACUUCUAGCUCGGA	611	3835	UCCGAGCUAGAAGUGGACA	936
3835	AACUUACUGUGUAAAUAAA	612	3835	AACUUACUGUGUAAAUAAA	612	3853	UUUAUUUACACAGUAAGUU	937
3853	ACUUUCAGAACUGCUACCA	613	3853	ACUUUCAGAACUGCUACCA	613	3871	UGGUAGCAGUUCUGAAAGU	938
3871	AUGAAGUGAAAAUGCCACA	614	3871	AUGAAGUGAAAAUGCCACA	614	3889	UGUGGCAUUUUCACUUCAU	939
3889	AUUUUGCUUUAUAAUUUCU	615	3889	AUUUUGCUUUAUAAUUUCU	615	3907	AGAAAUUAUAAAGCAAAAU	940
3907	UACCCAUGUUGGGAAAAAC	616	3907	UACCCAUGUUGGGAAAAAC	616	3925	GUUUUUCCCAACAUGGGUA	941
3925	CUGGCUUUUUCCCAGCCCU	617	3925	CUGGCUUUUUCCCAGCCCU	617	3943	AGGGCUGGGAAAAAGCCAG	942
3943	UUUCCAGGGCAUAAAACUC	618	3943	UUUCCAGGGCAUAAAACUC	618	3961	GAGUUUUAUGCCCUGGAAA	943
3961	CAACCCCUUCGAUAGCAAG	619	3961	CAACCCCUUCGAUAGCAAG	619	3979	CUUGCUAUCGAAGGGUUUG	944
3979	GUCCCAUCAGCCUAUUAUU	620	3979	GUCCCAUCAGCCUAUUAUU	620	3997	AAUAAUAGGCUGAUGGGAC	945
3997	UUUUUUAAAGAAAACUUGC	621	3997	UUUUUUAAAGAAAACUUGC	621	4015	GCAAGUUUUCUUUAAAAAA	946
4015	CACUUGUUUUUCUUUUUAC	622	4015	CACUUGUUUUUCUUUUUAC	622	4033	GUAAAAAGAAAAACAAGUG	947
4033	CAGUUACUUCCUUCCCUGCC	623	4033	CAGUUACUUCCUUCCCUGCC	623	4051	GGCAGGAAGGAAGUAACUG	948
4051	CCCAAAAUUAUAAACUCUA	624	4051	CCCAAAAUUAUAAACUCUA	624	4069	UAGAGUUUAUAAUUUUGGG	949
4069	AAGUGUAAAAAAAAAGUCUU	625	4069	AAGUGUAAAAAAAAAGUCUU	625	4087	AAGACUUUUUUUUUACACUU	950
4087	UAACAACAGCUUCUUGCUU	626	4087	UAACAACAGCUUCUUGCUU	626	4105	AAGCAAGAAGCUGUUGUUA	951
4105	UGUAAAAAUAUGUAUUAUA	627	4105	UGUAAAAAUAUGUAUUAUA	627	4123	UAUAAUACAUAUUUUUACA	952
4123	ACAUCUGUAUUUUUAAAUU	628	4123	ACAUCUGUAUUUUUAAAUU	628	4141	AAUUUAAAAAAUACAGAUGU	953
4141	UCUGCUCCUGAAAAAUGAC	629	4141	UCUGCUCCUGAAAAAUGAC	629	4159	GUCAUUUUUCAGGAGCAGA	954
4159	CUGUCCCAUUCUCCACUCA	630	4159	CUGUCCCAUUCUCCACUCA	630	4177	UGAGUGGAGAAUGGGACAG	955
4177	ACUGCAUUUGGGGCCUUUC	631	4177	ACUGCAUUUGGGGCCUUUC	631	4195	GAAAGGCCCCAAAUGCAGU	956

4195	CCCAUUGGUCUGCAUGUCU	632	4195	CCCAUUGGUCUGCAUGUCU	632	4213	AGACAUGCAGACCAAUGGG	957
4213	UUUUAUCAUUGCAGGCCAG	633	4213	UUUUAUCAUUGCAGGCCAG	633	4231	CUGGCCUGCAAUGAUAAAA	958
4231	GUGGACAGAGGGAGAAGGG	634	4231	GUGGACAGAGGGAGAAGGG	634	4249	CCCUUCUCCCCUCUGUCCAC	959
4249	GAGAACAGGGGUCGCCAAC	635	4249	GAGAACAGGGGUCGCCAAC	635	4267	GUUGGCGACCCCUGUUCUC	960
4267	CACUUGUGUUGCUUUCUGA	636	4267	CACUUGUGUUGCUUUCUGA	636	4285	UCAGAAAGCAACACAAGUG	961
4285	ACUGAUCCUGAACAAGAAA	637	4285	ACUGAUCCUGAACAAGAAA	637	4303	UUUCUUGUUCAGGAUCAGU	962
4303	AGAGUAACACUGAGGCGCU	638	4303	AGAGUAACACUGAGGCGCU	638	4321	AGCGCCUCAGUGUUACUCU	963
4321	UCGCUCCCAUGCACAACUC	639	4321	UCGCUCCCAUGCACAACUC	639	4339	GAGUUGUGCAUGGGAGCGA	964
4339	CUCCAAAACACUUAUCCUC	640	4339	CUCCAAAACACUUAUCCUC	640	4357	GAGGAUAAGUGUUUUGGAG	965
4357	CCUGCAAGAGUGGGCUUUC	641	4357	CCUGCAAGAGUGGGCUUUC	641	4375	GAAAGCCCACUCUUGCAGG	966
4375	CCAGGGUCUUUACUGGGAA	642	4375	CCAGGGUCUUUACUGGGAA	642	4393	UUCCCAGUAAAGACCCUGG	967
4393	AGCAGUUAAGCCCCCUCCU	643	4393	AGCAGUUAAGCCCCCUCCU	643	4411	AGGAGGGGGCUUAACUGCU	968
4411	UCACCCCUUCCUUUUUUCU	644	4411	UCACCCCUUCCUUUUUUCU	644	4429	AGAAAAAAGGAAGGGGUGA	969
4429	UUUCUUUACUCCUUUGGCU	645	4429	UUUCUUUACUCCUUUGGCU	645	4447	AGCCAAAGGAGUAAAGAAA	970
4447	UUCAAAGGAUUUUGGAAAA	646	4447	UUCAAAGGAUUUUGGAAAA	646	4465	UUUUCCAAAAUCCUUUGAA	971
4465	AGAAACAAUAUGCUUUACA	647	4465	AGAAACAAUAUGCUUUACA	647	4483	UGUAAAGCAUAUUGUUUCU	972
4483	ACUCAUUUUCAAUUUCUAA	648	4483	ACUCAUUUUCAAUUUCUAA	648	4501	UUAGAAAUUGAAAAUGAGU	973
4501	AAUUUGCAGGGGAUACUGA	649	4501	AAUUUGCAGGGGAUACUGA	649	4519	UCAGUAUCCCCUGCAAAUU	974
4519	AAAAAUACGGCAGGUGGCC	650	4519	AAAAAUACGGCAGGUGGCC	650	4537	GGCCACCUGCCGUAUUUUU	975
4537	CUAAGGCUGCUGUAAAGUU	651	4537	CUAAGGCUGCUGUAAAGUU	651	4555	AACUUUACAGCAGCCUUAG	976
4555	UGAGGGGAGAGGAAAUCUU	652	4555	UGAGGGGAGAGGAAAUCUU	652	4573	AAGAUUUCCUCUCCCCUCA	977
4573	UAAGAUUACAAGAUAAAAA	653	4573	UAAGAUUACAAGAUAAAAA	653	4591	UUUUUAUCUUGUAAUCUUA	978
4591	AACGAAUCCCCUAAACAAA	654	4591	AACGAAUCCCCUAAACAAA	654	4609	UUUGUUUAGGGGAUUCGUU	979
4609	AAAGAACAAUAGAACUGGU	655	4609	AAAGAACAAUAGAACUGGU	655	4627	ACCAGUUCUAUUGUUCUUU	980
4627	UCUUCCAUUUUGCCACCUU	656	4627	UCUUCCAUUUUGCCACCUU	656	4645	AAGGUGGCAAAAUGGAAGA	981
4645	UUCCUGUUCAUGACAGCUA	657	4645	UUCCUGUUCAUGACAGCUA	657	4663	UAGCUGUCAUGAACAGGAA	982
4663	ACUAACCUGGAGACAGUAA	658	4663	ACUAACCUGGAGACAGUAA	658	4681	UUACUGUCUCCAGGUUAGU	983
4681	ACAUUUCAUUAACCAAAGA	659	4681	ACAUUUCAUUAACCAAAGA	659	4699	UCUUUGGUUAAUGAAAUGU	984
4699	AAAGUGGGUCACCUGACCU	660	4699	AAAGUGGGUCACCUGACCU	660	4717	AGGUCAGGUGACCCACUUU	985
4717	UCUGAAGAGCUGAGUACUC	661	4717	UCUGAAGAGCUGAGUACUC	661	4735	GAGUACUCAGCUCUUCAGA	986
4735	CAGGCCACUCCAAUCACCC	662	4735	CAGGCCACUCCAAUCACCC	662	4753	GGGUGAUUGGAGUGGCCUG	987
4753	CUACAAGAUGCCAAGGAGG	663	4753	CUACAAGAUGCCAAGGAGG	663	4771	CCUCCUUGGCAUCUUGUAG	988
4771	GUCCCAGGAAGUCCAGCUC	664	4771	GUCCCAGGAAGUCCAGCUC	664	4789	GAGCUGGACUUCCUGGGAC	989
4789	CCUUAAACUGACGCUAGUC	665	4789	CCUUAAACUGACGCUAGUC	665	4807	GACUAGCGUCAGUUUAAGG	990

4807	CAAUAAACCUGGGCAAGUG	666	4807	CAAUAAACCUGGGCAAGUG	666	4825	CACUUGCCCAGGUUUAUUG	991
4825	GAGGCAAGAGAAAUGAGGA	667	4825	GAGGCAAGAGAAAUGAGGA	667	4843	UCCUCAUUUCUCUUGCCUC	992
4843	AAGAAUCCAUCUGUGAGGU	668	4843	AAGAAUCCAUCUGUGAGGU	668	4861	ACCUCACAGAUGGAUUCUU	993
4861	UGACAGGCAAGGAUGAAAG	669	4861	UGACAGGCAAGGAUGAAAG	669	4879	CUUUCAUCCUUGCCUGUCA	994
4879	GACAAAGAAGGAAAAGAGU	670	4879	GACAAAGAAGGAAAAGAGU	670	4897	ACUCUUUUCCCUUCUUUGUC	995
4897	UAUCAAAGGCAGAAAGGAG	671	4897	UAUCAAAGGCAGAAAGGAG	671	4915	CUCCUUUCUGCCUUUGAUA	996
4915	GAUCAUUUAGUUGGGUCUG	672	4915	GAUCAUUUAGUUGGGUCUG	672	4933	CAGACCCAACUAAAUGAUC	997
4933	GAAAGGAAAAGUCUUUGCU	673	4933	GAAAGGAAAAGUCUUUGCU	673	4951	AGCAAAGACUUUUCCUUUUC	998
4951	UAUCCGACAUGUACUGCUA	674	4951	UAUCCGACAUGUACUGCUA	674	4969	UAGCAGUACAUGUCGGAUA	999
4969	AGUACCUGUAAGCAUUUUA	675	4969	AGUACCUGUAAGCAUUUUA	675	4987	UAAAAUGCUUACAGGUACU	1000
4987	AGGUCCCAGAAUGGAAAAA	676	4987	AGGUCCCAGAAUGGAAAAA	676	5005	UUUUUCCAUUCUGGGACCU	1001
5005	AAAAAUCAGCUAUUGGUAA	677	5005	AAAAAUCAGCUAUUGGUAA	677	5023	UUACCAAUAGCUGAUUUUU	1002
5023	AUAUAAUAAUGUCCUUUCC	678	5023	AUAUAAUAAUGUCCUUUCC	678	5041	GGAAAGGACAUUAUUAUAU	1003
5041	CCUGGAGUCAGUUUUUUUA	679	5041	CCUGGAGUCAGUUUUUUUA	679	5059	UAAAAAAACUGACUCCAGG	1004
5059	AAAAAGUUAACUCUUAGUU	680	5059	AAAAAGUUAACUCUUAGUU	680	5077	AACUAAGAGUUAACUUUUU	1005
5077	UUUUACUUGUUUAAUUCUA	681	5077	UUUUACUUGUUUAAUUCUA	681	5095	UAGAAUUAAACAAGUAAAA	1006
5095	AAAAGAGAAGGGAGCUGAG	682	5095	AAAAGAGAAGGGAGCUGAG	682	5113	CUCAGCUCCCUUCUCUUUU	1007
5113	GGCCAUUCCCUGUAGGAGU	683	5113	GGCCAUUCCCUGUAGGAGU	683	5131	ACUCCUACAGGGAAUGGCC	1008
5131	UAAAGAUAAAAGGAUAGGA	684	5131	UAAAGAUAAAAGGAUAGGA	684	5149	UCCUAUCCUUUUAUCUUUA	1009
5149	AAAAGAUUCAAAGCUCUAA	685	5149	AAAAGAUUCAAAGCUCUAA	685	5167	UUAGAGCUUUGAAUCUUUU	1010
5167	AUAGAGUCACAGCUUUCCC	686	5167	AUAGAGUCACAGCUUUCCC	686	5185	GGGAAAGCUGUGACUCUAU	1011
5185	CAGGUAUAAAACCUAAAAU	687	5185	CAGGUAUAAAACCUAAAAU	687	5203	AUUUUAGGUUUUAUACCUG	1012
5203	UUAAGAAGUACAAUAAGCA	688	5203	UUAAGAAGUACAAUAAGCA	688	5221	UGCUUAUUGUACUCUUCUAA	1013
5221	AGAGGUGGAAAAUGAUCUA	689	5221	AGAGGUGGAAAAUGAUCUA	689	5239	UAGAUCAUUUUCCACCUCU	1014
5239	AGUUCCUGAUAGCUACCCA	690	5239	AGUUCCUGAUAGCUACCCA	690	5257	UGGGUAGCUAUCAGGAACU	1015
5257	ACAGAGCAAGUGAUUUAUA	691	5257	ACAGAGCAAGUGAUUUAUA	691	5275	UAUAAAUCACUUGCUCUGU	1016
5275	AAAUUUGAAAUCCAAACUA	692	5275	AAAUUUGAAAUCCAAACUA	692	5293	UAGUUUGGAUUUCAAAUUU	1017
5293	ACUUUCUUAAUAUCACUUU	693	5293	ACUUUCUUAAUAUCACUUU	693	5311	AAAGUGAUAUUAAGAAAGU	1018
5311	UGGUCUCCAUUUUUCCCAG	694	5311	UGGUCUCCAUUUUUCCCAG	694	5329	CUGGGAAAAAUGGAGACCA	1019
5329	GGACAGGAAAUAUGUCCCC	695	5329	GGACAGGAAAUAUGUCCCC	695	5347	GGGGACAUAUUUCCUGUCC	1020
5347	CCCCUAACUUUCUUGCUUC	696	5347	CCCCUAACUUUCUUGCUUC	696	5365	GAAGCAAGAAAGUUAGGGG	1021
5365	CAAAAAUUAAAAAUCCAGCA	697	5365	CAAAAAUUAAAAAUCCAGCA	697	5383	UGCUGGAUUUUAAUUUUUG	1022
5383	AUCCCAAGAUCAUUCUACA	698	5383	AUCCCAAGAUCAUUCUACA	698	5401	UGUAGAAUGAUCUUGGGAU	1023
5401	AAGUAAUUUUGCACAGACA	699	5401	AAGUAAUUUUGCACAGACA	699	5419	UGUCUGUGCAAAAUUACUU	1024

5419	AUCUCCUCACCCCAGUGCC	700	5419	AUCUCCUCACCCCAGUGCC	700	5437	GGCACUGGGGUGAGGAGAU	1025
5437	CUGUCUGGAGCUCACCCAA	701	5437	CUGUCUGGAGCUCACCCAA	701	5455	UUGGGUGAGCUCCAGACAG	1026
5455	AGGUCACCAAACAACUUGG	702	5455	AGGUCACCAAACAACUUGG	702	5473	CCAAGUUGUUUGGUGACCU	1027
5473	GUUGUGAACCAACUGCCUU	703	5473	GUUGUGAACCAACUGCCUU	703	5491	AAGGCAGUUGGUUCACAAC	1028
5491	UAACCUUCUGGGGGAGGGG	704	5491	UAACCUUCUGGGGGAGGGG	704	5509	CCCCUCCCCCCAGAAGGUUA	1029
5509	GGAUUAGCUAGACUAGGAG	705	5509	GGAUUAGCUAGACUAGGAG	705	5527	CUCCUAGUCUAGCUAAUCC	1030
5527	GACCAGAAGUGAAUGGGAA	706	5527	GACCAGAAGUGAAUGGGAA	706	5545	UUCCCAUUCACUUCUGGUC	1031
5545	AAGGGUGAGGACUUCACAA	707	5545	AAGGGUGAGGACUUCACAA	707	5563	UUGUGAAGUCCUCACCCUU	1032
5563	AUGUUGGCCUGUCAGAGCU	708	5563	AUGUUGGCCUGUCAGAGCU	708	5581	AGCUCUGACAGGCCAACAU	1033
5581	UUGAUUAGAAGCCAAGACA	709	5581	UUGAUUAGAAGCCAAGACA	709	5599	UGUCUUGGCUUCUAAUCAA	1034
5599	AGUGGCAGCAAAGGAAGAC	710	5599	AGUGGCAGCAAAGGAAGAC	710	5617	GUCUUCCUUUGCUGCCACU	1035
5617	CUUGGCCCAGGAAAAACCU	711	5617	CUUGGCCCAGGAAAAACCU	711	5635	AGGUUUUUCCCUGGGCCAAG	1036
5635	UGUGGGUUGUGCUAAUUUC	712	5635	UGUGGGUUGUGCUAAUUUC	712	5653	GAAAUUAGCACAACCCACA	1037
5653	CUGUCCAGAAAAUAGGGUG	713	5653	CUGUCCAGAAAAUAGGGUG	713	5671	CACCCUAUUUUCUGGACAG	1038
5671	GGACAGAAGCUUGUGGGGU	714	5671	GGACAGAAGCUUGUGGGGU	714	5689	ACCCCACAAGCUUCUGUCC	1039
5689	UGCAUGGAGGAAUUGGGAC	715	5689	UGCAUGGAGGAAUUGGGAC	715	5707	GUCCCAAUUCCUCCAUGCA	1040
5707	CCUGGUUAUGUUGUUAUUC	716	5707	CCUGGUUAUGUUGUUAUUC	716	5725	GAAUAACAACAUAACCAGG	1041
5725	CUCGGACUGUGAAUUUUGG	717	5725	CUCGGACUGUGAAUUUUGG	717	5743	CCAAAAUUCACAGUCCGAG	1042
5743	GUGAUGUAAAACAGAAUAU	718	5743	GUGAUGUAAAACAGAAUAU	718	5761	AUAUUCUGUUUUACAUCAC	1043
5761	UUCUGUAAACCUAAUGUCU	719	5761	UUCUGUAAACCUAAUGUCU	719	5779	AGACAUUAGGUUUACAGAA	1044
5779	UGUAUAAAUAAUGAGCGUU	720	5779	UGUAUAAAUAAUGAGCGUU	720	5797	AACGCUCAUUAUUUAUACA	1045
5797	UAACACAGUAAAAUAUUCA	721	5797	UAACACAGUAAAAUAUUCA	721	5815	UGAAUAUUUUACUGUGUUA	1046
5815	AAUAAGAAGUCAAAAAAAA	722	5815	AAUAAGAAGUCAAAAAAAA	722	5833	UUUUUUUUGACUUCUUAUU	1047
5821	AAGUCAAAAAAAAAAAAAA	723	5821	AAGUCAAAAAAAAAAAAAA	723	5839	UUUUUUUUUUUUUUGACUU	1048

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Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
3	GACAGAGUUACCUGCACCG	1049	3	GACAGAGUUACCUGCACCG	1049	21	CGGUGCAGGUAACUCUGUC	1132
21	GUUGUCCUACUUCCAGAAU	1050	21	GUUGUCCUACUUCCAGAAU	1050	39	AUUCUGGAAGUAGGACAAC	1133
39	UGCACAGAUGUCUGAGGAC	1051	39	UGCACAGAUGUCUGAGGAC	1051	57	GUCCUCAGACAUCUGUGCA	1134
57	CAACCACCUGAGCAAUACU	1052	57	CAACCACCUGAGCAAUACU	1052	75	AGUAUUGCUCAGGUGGUUG	1135
75	UAAUGACAAUAGAGAACGG	1053	75	UAAUGACAAUAGAGAACGG	1053	93	CCGUUCUCUAUUGUCAUUA	1136
93	GCAGGAGCACAACGACAGA	1054	93	GCAGGAGCACAACGACAGA	1054	111	UCUGUCGUUGUGCUCCUGC	1137
111	ACGGAGCCUUGGCCACCCU	1055	111	ACGGAGCCUUGGCCACCCU	1055	129	AGGGUGGCCAAGGCUCCGU	1138
129	UGAGCCAUUAUCUAAUGGA	1056	129	UGAGCCAUUAUCUAAUGGA	1056	147	UCCAUUAGAUAAUGGCUCA	1139
147	ACGACCCCAGGGUAACUCC	1057	147	ACGACCCCAGGGUAACUCC	1057	165	GGAGUUACCCUGGGGUCGU	1140
165	CCGGCAGGUGGUGGAGCAA	1058	165	CCGGCAGGUGGUGGAGCAA	1058	183	UUGCUCCCACCACCUGCCGG	1141
183	AGAUGAGGAAGAAGAUGAG	1059	183	AGAUGAGGAAGAAGAUGAG	1059	201	CUCAUCUUCUUCCCUCAUCU	1142
201	GGAGCUGACAUUGAAAUAU	1060	201	GGAGCUGACAUUGAAAUAU	1060	219	AUAUUUCAAUGUCAGCUCC	1143
219	UGGCGCCAAGCAUGUGAUC	1061	219	UGGCGCCAAGCAUGUGAUC	1061	237	GAUCACAUGCUUGGCGCCA	1144
237	CAUGCUCUUUGUCCCUGUG	1062	237	CAUGCUCUUUGUCCCUGUG	1062	255	CACAGGGACAAAGAGCAUG	1145
255	GACUCUCUGCAUGGUGGUG	1063	255	GACUCUCUGCAUGGUGGUG	1063	273	CACCACCAUGCAGAGAGUC	1146
273	GGUCGUGGCUACCAUUAAG	1064	273	GGUCGUGGCUACCAUUAAG	1064	291	CUUAAUGGUAGCCACGACC	1147
291	GUCAGUCAGCUUUUAUACC	1065	291	GUCAGUCAGCUUUUAUACC	1065	309	GGUAUAAAAGCUGACUGAC	1148
309	CCGGAAGGAUGGGCAGCUA	1066	309	CCGGAAGGAUGGGCAGCUA	1066	327	UAGCUGCCCAUCCUUCCGG	1149
327	AAUCUAUACCCCAUUCACA	1067	327	AAUCUAUACCCCAUUCACA	1067	345	UGUGAAUGGGGUAUAGAUU	1150
345	AGAAGAUACCGAGACUGUG	1068	345	AGAAGAUACCGAGACUGUG	1068	363	CACAGUCUCGGUAUCUUCU	1151
363	GGGCCAGAGAGCCCUGCAC	1069	363	GGGCCAGAGAGCCCUGCAC	1069	381	GUGCAGGGCUCUCUGGCCC	1152
381	CUCAAUUCUGAAUGCUGCC	1070	381	CUCAAUUCUGAAUGCUGCC	1070	399	GGCAGCAUUCAGAAUUGAG	1153
399	CAUCAUGAUCAGUGUCAUU	1071	399	CAUCAUGAUCAGUGUCAUU	1071	417	AAUGACACUGAUCAUGAUG	1154
417	UGUUGUCAUGACUAUCCUC	1072	417	UGUUGUCAUGACUAUCCUC	1072	435	GAGGAUAGUCAUGACAACA	1155
435	CCUGGUGGUUCUGUAUAAA	1073	435	CCUGGUGGUUCUGUAUAAA	1073	453	UUUAUACAGAACCACCAGG	1156
453	AUACAGGUGCUAUAAGGUC	1074	453	AUACAGGUGCUAUAAGGUC	1074	471	GACCUUAUAGCACCUGUAU	1157
471	CAUCCAUGCCUGGCUUAUU	1075	471	CAUCCAUGCCUGGCUUAUU	1075	489	AAUAAGCCAGGCAUGGAUG	1158
489	UAUAUCAUCUCUAUUGUUG	1076	489	UAUAUCAUCUCUAUUGUUG	1076	507	CAACAAUAGAGAUGAUAUA	1159
507	GCUGUUCUUUUUUUUCAUUC	1077	507	GCUGUUCUUUUUUUUCAUUC	1077	525	GAAUGAAAAAAAGAACAGC	1160
525	CAUUUACUUGGGGGGAAGUG	1078	525	CAUUUACUUGGGGGGAAGUG	1078	543	CACUUCCCCCCAAGUAAAUG	1161
543	GUUUAAAACCUAUAACGUU	1079	543	GUUUAAAACCUAUAACGUU	1079	561	AACGUUAUAGGUUUUAAAC	1162

561	UGCUGUGGACUACAUUACU	1080	561	UGCUGUGGACUACAUUACU	1080	579	AGUAAUGUAGUCCACAGCA	1163
579	UGUUGCACUCCUGAUCUGG	1081	579	UGUUGCACUCCUGAUCUGG	1081	597	CCAGAUCAGGAGUGCAACA	1164
597	GAAUUUUGGUGUGGUGGGA	1082	597	GAAUUUUGGUGUGGUGGGA	1082	615	UCCCACCACACCAAAAUUC	1165
615	AAUGAUUUCCAUUCACUGG	1083	615	AAUGAUUUCCAUUCACUGG	1083	633	CCAGUGAAUGGAAAUCAUU	1166
633	GAAAGGUCCACUUCGACUC	1084	633	GAAAGGUCCACUUCGACUC	1084	651	GAGUCGAAGUGGACCUUUC	1167
651	CCAGCAGGCAUAUCUCAUU	1085	651	CCAGCAGGCAUAUCUCAUU	1085	669	AAUGAGAUAUGCCUGCUGG	1168
669	UAUGAUUAGUGCCCUCAUG	1086	669	UAUGAUUAGUGCCCUCAUG	1086	687	CAUGAGGGCACUAAUCAUA	1169
687	GGCCCUGGUGUUUAUCAAG	1087	687	GGCCCUGGUGUUUAUCAAG	1087	705	CUUGAUAAACACCAGGGCC	1170
705	GUACCUCCCUGAAUGGACU	1088	705	GUACCUCCCUGAAUGGACU	1088	723	AGUCCAUUCAGGGAGGUAC	1171
723	UGCGUGGCUCAUCUUGGCU	1089	723	UGCGUGGCUCAUCUUGGCU	1089	741	AGCCAAGAUGAGCCACGCA	1172
741	UGUGAUUUCGGUAUAUGAU	1090	741	UGUGAUUUCGGUAUAUGAU	1090	759	AUCAUAUACCGAAAUCACA	1173
759	UUUAGUGGCUGUUUUUGUGU	1091	759	UUUAGUGGCUGUUUUUGUGU	1091	777	ACACAAAACAGCCACUAAA	1174
777	UCCGAAAGGUCCACUUCGU	1092	777	UCCGAAAGGUCCACUUCGU	1092	795	ACGAAGUGGACCUUUCGGA	1175
795	UAUGCUGGUUGAAACAGCU	1093	795	UAUGCUGGUUGAAACAGCU	1093	813	AGCUGUUUCAACCAGCAUA	1176
813	UCAGGAGAGAAAUGAAACG	1094	813	UCAGGAGAGAAAUGAAACG	1094	831	CGUUUCAUUUCUCUCCUGA	1177
831	GCUUUUUCCAGCUCUCAUU	1095	831	GCUUUUUCCAGCUCUCAUU	1095	849	AAUGAGAGCUGGAAAAAGC	1178
849	UUACUCCUCAACAAUGGUG	1096	849	UUACUCCUCAACAAUGGUG	1096	867	CACCAUUGUUGAGGAGUAA	1179
867	GUGGUUGGUGAAUAUGGCA	1097	867	GUGGUUGGUGAAUAUGGCA	1097	885	UGCCAUAUUCACCAACCAC	1180
885	AGAAGGAGACCCGGAAGCU	1098	885	AGAAGGAGACCCGGAAGCU	1098	903	AGCUUCCGGGUCUCCUUCU	1181
903	UCAAAGGAGAGUAUCCAAA	1099	903	UCAAAGGAGAGUAUCCAAA	1099	921	UUUGGAUACUCUCCUUUGA	1182
921	AAAUUCCAAGUAUAAUGCA	1100	921	AAAUUCCAAGUAUAAUGCA	1100	939	UGCAUUAUACUUGGAAUUU	1183
939	AGAAAGAGCCUGUCUGCCU	1101	939	AGAAAGAGCCUGUCUGCCU	1101	957	AGGCAGACAGGCUCUUUCU	1184
957	UCCUGCUGCCAUCAACCUG	1102	957	UCCUGCUGCCAUCAACCUG	1102	975	CAGGUUGAUGGCAGCAGGA	1185
975	GCUGUCUAUAGCUCCCAUG	1103	975	GCUGUCUAUAGCUCCCAUG	1103	993	CAUGGGAGCUAUAGACAGC	1186
993	GGCACCCAGGCUGUUCAUG	1104	993	GGCACCCAGGCUGUUCAUG	1104	1011	CAUGAACAGCCUGGGUGCC	1187
1011	GCCAAAGGGUGCCUGCAGG	1105	1011	GCCAAAGGGUGCCUGCAGG	1105	1029	CCUGCAGGCACCCUUUGGC	1188
1029	GCCCACGGCACAGAAAGGG	1106	1029	GCCCACGGCACAGAAAGGG	1106	1047	CCCUUUCUGUGCCGUGGGC	1189
1047	GAGUCACAAGACACUGUUG	1107	1047	GAGUCACAAGACACUGUUG	1107	1065	CAACAGUGUCUUGUGACUC	1190
1065	GCAGAGAAUGAUGAUGGCG	1108	1065	GCAGAGAAUGAUGAUGGCG	1108	1083	CGCCAUCAUCAUUCUCUGC	1191
1083	GGGUUCAGUGAGGAAUGGG	1109	1083	GGGUUCAGUGAGGAAUGGG	1109	1101	CCCAUUCCUCACUGAACCC	1192
1101	GAAGCCCAGAGGGACAGUC	1110	1101	GAAGCCCAGAGGGACAGUC	1110	1119	GACUGUCCCCUCUGGGCUUC	1193
1119	CAUCUAGGGCCUCAUCGCU	1111	1119	CAUCUAGGGCCUCAUCGCU	1111	1137	AGCGAUGAGGCCCUAGAUG	1194
1137	UCUACACCUGAGUCACGAG	1112	1137	UCUACACCUGAGUCACGAG	1112	1155	CUCGUGACUCAGGUGUAGA	1195
1155	GCUGCUGUCCAGGAACUUU	1113	1155	GCUGCUGUCCAGGAACUUU	1113	1173	AAAGUUCCUGGACAGCAGC	1196

1173	UCCAGCAGUAUCCUCGCUG	1114	1173	UCCAGCAGUAUCCUCGCUG	1114	1191	CAGCGAGGAUACUGCUGGA	1197
1191	GGUGAAGACCCAGAGGAAA	1115	1191	GGUGAAGACCCAGAGGAAA	1115	1209	UUUCCUCUGGGUCUUCACC	1198
1209	AGGGGAGUAAAACUUGGAU	1116	1209	AGGGGAGUAAAACUUGGAU	1116	1227	AUCCAAGUUUUACUCCCCU	1199
1227	UUGGGAGAUUUCAUUUUCU	1117	1227	UUGGGAGAUUUCAUUUUCU	1117	1245	AGAAAAUGAAAUCUCCCAA	1200
1245	UACAGUGUUCUGGUUGGUA	1118	1245	UACAGUGUUCUGGUUGGUA	1118	1263	UACCAACCAGAACACUGUA	1201
1263	AAAGCCUCAGCAACAGCCA	1119	1263	AAAGCCUCAGCAACAGCCA	1119	1281	UGGCUGUUGCUGAGGCUUU	1202
1281	AGUGGAGACUGGAACACAA	1120	1281	AGUGGAGACUGGAACACAA	1120	1299	UUGUGUUCCAGUCUCCACU	1203
1299	ACCAUAGCCUGUUUCGUAG	1121	1299	ACCAUAGCCUGUUUCGUAG	1121	1317	CUACGAAACAGGCUAUGGU	1204
1317	GCCAUAUUAAUUGGUUUGU	1122	1317	GCCAUAUUAAUUGGUUUGU	1122	1335	ACAAACCAAUUAAUAUGGC	1205
1335	UGCCUUACAUUAUUACUCC	1123	1335	UGCCUUACAUUAUUACUCC	1123	1353	GGAGUAAUAAUGUAAGGCA	1206
1353	CUUGCCAUUUUCAAGAAAG	1124	1353	CUUGCCAUUUUCAAGAAAG	1124	1371	CUUUCUUGAAAAUGGCAAG	1207
1371	GCAUUGCCAGCUCUUCCAA	1125	1371	GCAUUGCCAGCUCUUCCAA	1125	1389	UUGGAAGAGCUGGCAAUGC	1208
1389	AUCUCCAUCACCUUUGGGC	1126	1389	AUCUCCAUCACCUUUGGGC	1126	1407	GCCCAAAGGUGAUGGAGAU	1209
1407	CUUGUUUUCUACUUUGCCA	1127	1407	CUUGUUUUCUACUUUGCCA	1127	1425	UGGCAAAGUAGAAAACAAG	1210
1425	ACAGAUUAUCUUGUACAGC	1128	1425	ACAGAUUAUCUUGUACAGC	1128	1443	GCUGUACAAGAUAAAUCUGU	1211
1443	CCUUUUAUGGACCAAUUAG	1129	1443	CCUUUUAUGGACCAAUUAG	1129	1461	CUAAUUGGUCCAUAAAAGG	1212
1461	GCAUUCCAUCAAUUUUAUA	1130	1461	GCAUUCCAUCAAUUUUAUA	1130	1479	UAUAAAAUUGAUGGAAUGC	1213
1464	UUCCAUCAAUUUUAUAUCU	1131	1464	UUCCAUCAAUUUUAUAUCU	1131	1482	AGAUAUAAAAUUGAUGGAA	1214

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Pos	Seq	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
3	AGCGGCGGCGGAGCAGGCA	1215	3	AGCGGCGGCGGAGCAGGCA	1215	21	UGCCUGCUCCGCCGCCGCU	1339
21	AUUUCCAGCAGUGAGGAGA	1216	21	AUUUCCAGCAGUGAGGAGA	1216	39	UCUCCUCACUGCUGGAAAU	1340
39	ACAGCCAGAAGCAAGCUAU	1217	39	ACAGCCAGAAGCAAGCUAU	1217	57	AUAGCUUGCUUCUGGCUGU	1341
57	UUGGAGCUGAAGGAACCUG	1218	57	UUGGAGCUGAAGGAACCUG	1218	75	CAGGUUCCUUCAGCUCCAA	1342
75	GAGACAGAAGCUAGUCCCC	1219	75	GAGACAGAAGCUAGUCCCC	1219	93	GGGGACUAGCUUCUGUCUC	1343
93	CCCUCUGAAUUUUACUGAU	1220	93	CCCUCUGAAUUUUACUGAU	1220	111	AUCAGUAAAAUUCAGAGGG	1344
111	UGAAGAAACUGAGGCCACA	1221	111	UGAAGAAACUGAGGCCACA	1221	129	UGUGGCCUCAGUUUCUUCA	1345
129	AGAGCUAAAGUGACUUUUC	1222	129	AGAGCUAAAGUGACUUUUC	1222	147	GAAAAGUCACUUUAGCUCU	1346
147	CCCAAGGUCGCCCAGCGAG	1223	147	CCCAAGGUCGCCCAGCGAG	1223	165	CUCGCUGGGGCGACCUUGGG	1347
165	GGACGUGGGACUUCUCAGA	1224	165	GGACGUGGGACUUCUCAGA	1224	183	UCUGAGAAGUCCCACGUCC	1348
183	ACGUCAGGAGAGUGAUGUG	1225	183	ACGUCAGGAGAGUGAUGUG	1225	201	CACAUCACUCUCCUGACGU	1349
201	GAGGGAGCUGUGUGACCAU	1226	201	GAGGGAGCUGUGUGACCAU	1226	219	AUGGUCACACAGCUCCCUC	1350
219	UAGAAAGUGACGUGUUAAA	1227	219	UAGAAAGUGACGUGUUAAA	1227	237	UUUAACACGUCACUUUCUA	1351
237	AAACCAGCGCUGCCCUCUU	1228	237	AAACCAGCGCUGCCCUCUU	1228	255	AAGAGGGCAGCGCUGGUUU	1352
255	UUGAAAGCCAGGGAGCAUC	1229	255	UUGAAAGCCAGGGAGCAUC	1229	273	GAUGCUCCCCUGGCUUUCAA	1353
273	CAUUCAUUUAGCCUGCUGA	1230	273	CAUUCAUUUAGCCUGCUGA	1230	291	UCAGCAGGCUAAAUGAAUG	1354
291	AGAAGAAGAAACCAAGUGU	1231	291	AGAAGAAGAAACCAAGUGU	1231	309	ACACUUGGUUUCUUCUUCU	1355
309	UCCGGGAUUCAGACCUCUC	1232	309	UCCGGGAUUCAGACCUCUC	1232	327	GAGAGGUCUGAAUCCCGGA	1356
327	CUGCGGCCCCAAGUGUUCG	1233	327	CUGCGGCCCCAAGUGUUCG	1233	345	CGAACACUUGGGGCCGCAG	1357
345	GUGGUGCUUCCAGAGGCAG	1234	345	GUGGUGCUUCCAGAGGCAG	1234	363	CUGCCUCUGGAAGCACCAC	1358
363	GGGCUAUGCUCACAUUCAU	1235	363	GGGCUAUGCUCACAUUCAU	1235	381	AUGAAUGUGAGCAUAGCCC	1359
381	UGGCCUCUGACAGCGAGGA	1236	381	UGGCCUCUGACAGCGAGGA	1236	399	UCCUCGCUGUCAGAGGCCA	1360
399	AAGAAGUGUGUGAUGAGCG	1237	399	AAGAAGUGUGUGAUGAGCG	1237	417	CGCUCAUCACACACUUCUU	1361
417	GGACGUCCCUAAUGUCGGC	1238	417	GGACGUCCCUAAUGUCGGC	1238	435	GCCGACAUUAGGGACGUCC	1362
435	CCGAGAGCCCCACGCCGCG	1239	435	CCGAGAGCCCCACGCCGCG	1239	453	CGCGGCGUGGGGCUCUCGG	1363
453	GCUCCUGCCAGGAGGGCAG	1240	453	GCUCCUGCCAGGAGGGCAG	1240	471	CUGCCCUCCUGGCAGGAGC	1364
471	GGCAGGGCCCAGAGGAUGG	1241	471	GGCAGGGCCCAGAGGAUGG	1241	489	CCAUCCUCUGGGCCCUGCC	1365
489	GAGAGAACACUGCCCAGUG	1242	489	GAGAGAACACUGCCCAGUG	1242	507	CACUGGGCAGUGUUCUCUC	1366
507	GGAGAAGCCAGGAGAACGA	1243	507	GGAGAAGCCAGGAGAACGA	1243	525	UCGUUCUCCUGGCUUCUCC	1367
525	AGGAGGACGGUGAGGAGGA	1244	525	AGGAGGACGGUGAGGAGGA	1244	543	UCCUCCUCACCGUCCUCCU	1368
543	ACCCUGACCGCUAUGUCUG	1245	543	ACCCUGACCGCUAUGUCUG	1245	561	CAGACAUAGCGGUCAGGGU	1369

561	GUAGUGGGGUUCCCGGGCG	1246	561	GUAGUGGGGUUCCCGGGCG	1246	579	CGCCCGGGAACCCCACUAC	1370
579	GGCCGCCAGGCCUGGAGGA	1247	579	GGCCGCCAGGCCUGGAGGA	1247	597	UCCUCCAGGCCUGGCGGCC	1371
597	AAGAGCUGACCCUCAAAUA	1248	597	AAGAGCUGACCCUCAAAUA	1248	615	UAUUUGAGGGUCAGCUCUU	1372
615	ACGGAGCGAAGCACGUGAU	1249	615	ACGGAGCGAAGCACGUGAU	1249	633	AUCACGUGCUUCGCUCCGU	1373
633	UCAUGCUGUUUGUGCCUGU	1250	633	UCAUGCUGUUUGUGCCUGU	1250	651	ACAGGCACAAACAGCAUGA	1374
651	UCACUCUGUGCAUGAUCGU	1251	651	UCACUCUGUGCAUGAUCGU	1251	669	ACGAUCAUGCACAGAGUGA	1375
669	UGGUGGUAGCCACCAUCAA	1252	669	UGGUGGUAGCCACCAUCAA	1252	687	UUGAUGGUGGCUACCACCA	1376
687	AGUCUGUGCGCUUCUACAC	1253	687	AGUCUGUGCGCUUCUACAC	1253	705	GUGUAGAAGCGCACAGACU	1377
705	CAGAGAAGAAUGGACAGCU	1254	705	CAGAGAAGAAUGGACAGCU	1254	723	AGCUGUCCAUUCUUCUCUG	1378
723	UCAUCUACACGACAUUCAC	1255	723	UCAUCUACACGACAUUCAC	1255	741	GUGAAUGUCGUGUAGAUGA	1379
741	CUGAGGACACACCCUCGGU	1256	741	CUGAGGACACACCCUCGGU	1256	759	ACCGAGGGUGUGUCCUCAG	1380
759	UGGGCCAGCGCCUCCUCAA	1257	759	UGGGCCAGCGCCUCCUCAA	1257	777	UUGAGGAGGCGCUGGCCCA	1381
777	ACUCCGUGCUGAACACCCU	1258	777	ACUCCGUGCUGAACACCCU	1258	795	AGGGUGUUCAGCACGGAGU	1382
795	UCAUCAUGAUCAGCGUCAU	1259	795	UCAUCAUGAUCAGCGUCAU	1259	813	AUGACGCUGAUCAUGAUGA	1383
813	UCGUGGUUAUGACCAUCUU	1260	813	UCGUGGUUAUGACCAUCUU	1260	831	AAGAUGGUCAUAACCACGA	1384
831	UCUUGGUGGUGCUCUACAA	1261	831	UCUUGGUGGUGCUCUACAA	1261	849	UUGUAGAGCACCACCAAGA	1385
849	AGUACCGCUGCUACAAGUU	1262	849	AGUACCGCUGCUACAAGUU	1262	867	AACUUGUAGCAGCGGUACU	1386
867	UCAUCCAUGGCUGGUUGAU	1263	867	UCAUCCAUGGCUGGUUGAU	1263	885	AUCAACCAGCCAUGGAUGA	1387
885	UCAUGUCUUCACUGAUGCU	1264	885	UCAUGUCUUCACUGAUGCU	1264	903	AGCAUCAGUGAAGACAUGA	1388
903	UGCUGUUCCUCUUCACCUA	1265	903	UGCUGUUCCUCUUCACCUA	1265	921	UAGGUGAAGAGGAACAGCA	1389
921	AUAUCUACCUUGGGGAAGU	1266	921	AUAUCUACCUUGGGGAAGU	1266	939	ACUUCCCCAAGGUAGAUAU	1390
939	UGCUCAAGACCUACAAUGU	1267	939	UGCUCAAGACCUACAAUGU	1267	957	ACAUUGUAGGUCUUGAGCA	1391
957	UGGCCAUGGACUACCCCAC	1268	957	UGGCCAUGGACUACCCCAC	1268	975	GUGGGGUAGUCCAUGGCCA	1392
975	CCCUCUUGCUGACUGUCUG	1269	975	CCCUCUUGCUGACUGUCUG	1269	993	CAGACAGUCAGCAAGAGGG	1393
993	GGAACUUCGGGGCAGUGGG	1270	993	GGAACUUCGGGGCAGUGGG	1270	1011	CCCACUGCCCCGAAGUUCC	1394
1011	GCAUGGUGUGCAUCCACUG	1271	1011	GCAUGGUGUGCAUCCACUG	1271	1029	CAGUGGAUGCACACCAUGC	1395
1029	GGAAGGGCCCUCUGGUGCU	1272	1029	GGAAGGGCCCUCUGGUGCU	1272	1047	AGCACCAGAGGGCCCUUCC	1396
1047	UGCAGCAGGCCUACCUCAU	1273	1047	UGCAGCAGGCCUACCUCAU	1273	1065	AUGAGGUAGGCCUGCUGCA	1397
1065	UCAUGAUCAGUGCGCUCAU	1274	1065	UCAUGAUCAGUGCGCUCAU	1274	1083	AUGAGCGCACUGAUCAUGA	1398
1083	UGGCCCUAGUGUUCAUCAA	1275	1083	UGGCCCUAGUGUUCAUCAA	1275	1101	UUGAUGAACACUAGGGCCA	1399
1101	AGUACCUCCCAGAGUGGUC	1276	1101	AGUACCUCCCAGAGUGGUC	1276	1119	GACCACUCUGGGAGGUACU	1400
1119	CCGCGUGGGUCAUCCUGGG	1277	1119	CCGCGUGGGUCAUCCUGGG	1277	1137	CCCAGGAUGACCCACGCGG	1401
1137	GCGCCAUCUCUGUGUAUGA	1278	1137	GCGCCAUCUCUGUGUAUGA	1278	1155	UCAUACACAGAGAUGGCGC	1402
1155	AUCUCGUGGCUGUGCUGUG	1279	1155	AUCUCGUGGCUGUGCUGUG	1279	1173	CACAGCACAGCCACGAGAU	1403
1173	GUCCCAAAGGGCCUCUGAG	1280	1173	GUCCCAAAGGGCCUCUGAG	1280	1191	CUCAGAGGCCCUUUGGGAC	1404

1191	GAAUGCUGGUAGAAACUGC	1281	1191	GAAUGCUGGUAGAAACUGC	1281	1209	GCAGUUUCUACCAGCAUUC	1405
1209	CCCAGGAGAGAAAUGAGCC	1282	1209	CCCAGGAGAGAAAUGAGCC	1282	1227	GGCUCAUUUCUCUCCCUGGG	1406
1227	CCAUAUUCCCUGCCCCUGAU	1283	1227	CCAUAUUCCCUGCCCCUGAU	1283	1245	AUCAGGGCAGGGAAUAUGG	1407
1245	UAUACUCAUCUGCCAUGGU	1284	1245	UAUACUCAUCUGCCAUGGU	1284	1263	ACCAUGGCAGAUGAGUAUA	1408
1263	UGUGGACGGUUGGCAUGGC	1285	1263	UGUGGACGGUUGGCAUGGC	1285	1281	GCCAUGCCAACCGUCCACA	1409
1281	CGAAGCUGGACCCCUCCUC	1286	1281	CGAAGCUGGACCCCUCCUC	1286	1299	GAGGAGGGGUCCAGCUUCG	1410
1299	CUCAGGGUGCCCUCCAGCU	1287	1299	CUCAGGGUGCCCUCCAGCU	1287	1317	AGCUGGAGGGCACCCUGAG	1411
1317	UCCCCUACGACCCGGAGAU	1288	1317	UCCCCUACGACCCGGAGAU	1288	1335	AUCUCCGGGUCGUAGGGGA	1412
1335	UGGAAGAAGACUCCUAUGA	1289	1335	UGGAAGAAGACUCCUAUGA	1289	1353	UCAUAGGAGUCUUCUUCCA	1413
1353	ACAGUUUUGGGGAGCCUUC	1290	1353	ACAGUUUUGGGGAGCCUUC	1290	1371	GAAGGCUCCCCCAAAACUGU	1414
1371	CAUACCCCGAAGUCUUUGA	1291	1371	CAUACCCCGAAGUCUUUGA	1291	1389	UCAAAGACUUCGGGGUAUG	1415
1389	AGCCUCCCUUGACUGGCUA	1292	1389	AGCCUCCCUUGACUGGCUA	1292	1407	UAGCCAGUCAAGGGAGGCU	1416
1407	ACCCAGGGGAGGAGCUGGA	1293	1407	ACCCAGGGGAGGAGCUGGA	1293	1425	UCCAGCUCCUCCCCCUGGGU	1417
1425	AGGAAGAGGAGGAAAGGGG	1294	1425	AGGAAGAGGAGGAAAGGGG	1294	1443	CCCCUUUCCUCCUCUUCCU	1418
1443	GCGUGAAGCUUGGCCUCGG	1295	1443	GCGUGAAGCUUGGCCUCGG	1295	1461	CCGAGGCCAAGCUUCACGC	1419
1461	GGGACUUCAUCUUCUACAG	1296	1461	GGGACUUCAUCUUCUACAG	1296	1479	CUGUAGAAGAUGAAGUCCC	1420
1479	GUGUGCUGGUGGGCAAGGC	1297	1479	GUGUGCUGGUGGGCAAGGC	1297	1497	GCCUUGCCCACCAGCACAC	1421
1497	CGGCUGCCACGGGCAGCGG	1298	1497	CGGCUGCCACGGGCAGCGG	1298	1515	CCGCUGCCCGUGGCAGCCG	1422
1515	GGGACUGGAAUACCACGCU	1299	1515	GGGACUGGAAUACCACGCU	1299	1533	AGCGUGGUAUUCCAGUCCC	1423
1533	UGGCCUGCUUCGUGGCCAU	1300	1533	UGGCCUGCUUCGUGGCCAU	1300	1551	AUGGCCACGAAGCAGGCCA	1424
1551	UCCUCAUUGGCUUGUGUCU	1301	1551	UCCUCAUUGGCUUGUGUCU	1301	1569	AGACACAAGCCAAUGAGGA	1425
1569	UGACCCUCCUGCUUUGC	1302	1569	UGACCCUCCUGCUUUGC	1302	1587	GCAAGCAGCAGGAGGGUCA	1426
1587	CUGUGUUCAAGAAGGCGCU	1303	1587	CUGUGUUCAAGAAGGCGCU	1303	1605	AGCGCCUUCUUGAACACAG	1427
1605	UGCCCGCCCUCCCCCAUCUC	1304	1605	UGCCCGCCCUCCCCCAUCUC	1304	1623	GAGAUGGGGAGGGCGGGCA	1428
1623	CCAUCACGUUCGGGCUCAU	1305	1623	CCAUCACGUUCGGGCUCAU	1305	1641	AUGAGCCCGAACGUGAUGG	1429
1641	UCUUUUACUUCUCCCACGGA	1306	1641	UCUUUUACUUCUCCCACGGA	1306	1659	UCCGUGGAGAAGUAAAAGA	1430
1659	ACAACCUGGUGCGGCCGUU	1307	1659	ACAACCUGGUGCGGCCGUU	1307	1677	AACGGCCGCACCAGGUUGU	1431
1677	UCAUGGACACCCUGGCCUC	1308	1677	UCAUGGACACCCUGGCCUC	1308	1695	GAGGCCAGGGUGUCCAUGA	1432
1695	CCCAUCAGCUCUACAUCUG	1309	1695	CCCAUCAGCUCUACAUCUG	1309	1713	CAGAUGUAGAGCUGAUGGG	1433
1713	GAGGGACAUGGUGUGCCAC	1310	1713	GAGGGACAUGGUGUGCCAC	1310	1731	GUGGCACACCAUGUCCCUC	1434
1731	CAGGCUGCAAGCUGCAGGG	1311	1731	CAGGCUGCAAGCUGCAGGG	1311	1749	CCCUGCAGCUUGCAGCCUG	1435
1749	GAAUUUUCAUUGGAUGCAG	1312	1749	GAAUUUUCAUUGGAUGCAG	1312	1767	CUGCAUCCAAUGAAAAUUC	1436
1767	GUUGUAUAGUUUUACACUC	1313	1767	GUUGUAUAGUUUUACACUC	1313	1785	GAGUGUAAAACUAUACAAC	1437
1785	CUAGUGCCAUAUAUUUUUA	1314	1785	CUAGUGCCAUAUAUUUUUA	1314	1803	UAAAAAUAUAUGGCACUAG	1438
1803	AAGACUUUUCUUUCCUUAA	1315	1803	AAGACUUUUCUUUCCUUAA	1315	1821	UUAAGGAAAGAAAAGUCUU	1439

1821	AAAAAUAAAGUACGUGUUU	1316	1821	AAAAAUAAAGUACGUGUUU	1316	1839	AAACACGUACUUUAUUUUU	1440
1839	UACUUGGUGAGGAGGAGGC	1317	1839	UACUUGGUGAGGAGGAGGC	1317	1857	GCCUCCUCCUCACCAAGUA	1441
1857	CAGAACCAGCUCUUUGGUG	1318	1857	CAGAACCAGCUCUUUGGUG	1318	1875	CACCAAAGAGCUGGUUCUG	1442
1875	GCCAGCUGUUUCAUCACCA	1319	1875	GCCAGCUGUUUCAUCACCA	1319	1893	UGGUGAUGAAACAGCUGGC	1443
1893	AGACUUUGGCUCCCGCUUU	1320	1893	AGACUUUGGCUCCCGCUUU	1320	1911	AAAGCGGGAGCCAAAGUCU	1444
1911	UGGGGAGCGCCUCGCUUCA	1321	1911	UGGGGAGCGCCUCGCUUCA	1321	1929	UGAAGCGAGGCGCUCCCCA	1445
1929	ACGGACAGGAAGCACAGCA	1322	1929	ACGGACAGGAAGCACAGCA	1322	1947	UGCUGUGCUUCCUGUCCGU	1446
1947	AGGUUUAUCCAGAUGAACU	1323	1947	AGGUUUAUCCAGAUGAACU	1323	1965	AGUUCAUCUGGAUAAACCU	1447
1965	UGAGAAGGUCAGAUUAGGG	1324	1965	UGAGAAGGUCAGAUUAGGG	1324	1983	CCCUAAUCUGACCUUCUCA	1448
1983	GCGGGGAGAAGAGCAUCCG	1325	1983	GCGGGGAGAAGAGCAUCCG	1325	2001	CGGAUGCUCUUCUCCCCGC	1449
2001	GGCAUGAGGGCUGAGAUGC	1326	2001	GGCAUGAGGGCUGAGAUGC	1326	2019	GCAUCUCAGCCCUCAUGCC	1450
2019	CGCAAAGAGUGUGCUCGGG	1327	2019	CGCAAAGAGUGUGCUCGGG	1327	2037	CCCGAGCACACUCUUUGCG	1451
2037	GAGUGGCCCCUGGCACCUG	1328	2037	GAGUGGCCCCUGGCACCUG	1328	2055	CAGGUGCCAGGGGCCACUC	1452
2055	GGGUGCUCUGGCUGGAGAG	1329	2055	GGGUGCUCUGGCUGGAGAG	1329	2073	CUCUCCAGCCAGAGCACCC	1453
2073	GGAAAAGCCAGUUCCCUAC	1330	2073	GGAAAAGCCAGUUCCCUAC	1330	2091	GUAGGGAACUGGCUUUUCC	1454
2091	CGAGGAGUGUUCCCAAUGC	1331	2091	CGAGGAGUGUUCCCAAUGC	1331	2109	GCAUUGGGAACACUCCUCG	1455
2109	CUUUGUCCAUGAUGUCCUU	1332	2109	CUUUGUCCAUGAUGUCCUU	1332	2127	AAGGACAUCAUGGACAAAG	1456
2127	UGUUAUUUUAUUGCCUUUA	1333	2127	UGUUAUUUUAUUGCCUUUA	1333	2145	UAAAGGCAAUAAAAUAACA	1457
2145	AGAAACUGAGUCCUGUUCU	1334	2145	AGAAACUGAGUCCUGUUCU	1334	2163	AGAACAGGACUCAGUUUCU	1458
2163	UUGUUACGGCAGUCACACU	1335	2163	UUGUUACGGCAGUCACACU	1335	2181	AGUGUGACUGCCGUAACAA	1459
2181	UGCUGGGAAGUGGCUUAAU	1336	2181	UGCUGGGAAGUGGCUUAAU	1336	2199	AUUAAGCCACUUCCCAGCA	1460
2199	UAGUAAUAUCAAUAAAUAG	1337	2199	UAGUAAUAUCAAUAAAUAG	1337	2217	CUAUUUAUUGAUAUUACUA	1461
2216	AGAUGAGUCCUGUUAGAAA	1338	2216	AGAUGAGUCCUGUUAGAAA	1338	2234	UUUCUAACAGGACUCAUCU	1462

The 3'-ends of the Upper sequence and the Lower sequence of the siNA construct can include an overhang sequence, for example about 1, 2, 3, or 4 nucleotides in length, preferably 2 nucleotides in length, wherein the overhanging sequence of the lower sequence is optionally complementary to a portion of the target sequence. The upper sequence is also referred to as the sense strand, whereas the lower sequence is also referred to as the antisense strand. The upper and lower sequences in the Table can further comprise a chemical modification having Formulae I-VII or any combination thereof.

TABLE III: APP, BACE, PSEN1, PSEN2, SYNTHETIC MODIFIED siNA CONSTRUCTS

APP

Target Pos	Target	Seq ID	Cmpd#	Aliases	Sequence	Seq ID
791	CAGACUAUGCAGAUGGGAGUGAA	1463		APP:793U21 sense siNA	GACUAUGCAGAUGGGAGUGTT	1495
829	GUAGCAGAGGAGGAAGAAGUGGC	1464		APP:831U21 sense siNA	AGCAGAGGAGGAAGAAGUGTT	1496
851	CUGAGGUGGAAGAAGAAGAAGCC	1465		APP:853U21 sense siNA	GAGGUGGAAGAAGAAGAAGTT	1497
1356	AGAGAGAAUGUCCCAGGUCAUGA	1466		APP:1358U21 sense siNA	AGAGAAUGUCCCAGGUCAUTT	1498
1568	AGAACUACAUCACCGCUCUGCAG	1467		APP:1570U21 sense siNA	AACUACAUCACCGCUCUGCTT	1499
2012	AUUCUUUUGGGGCUGACUCUGUG	1468		APP:2014U21 sense siNA	UCUUUUGGGGCUGACUCUGTT	1500
2481	UGAAGUUGGACAGCAAAACCAUU	1469		APP:2483U21 sense siNA	AAGUUGGACAGCAAAACCATT	1501
2482	GAAGUUGGACAGCAAAACCAUUG	1470		APP:2484U21 sense siNA	AGUUGGACAGCAAAACCAUTT	1502
791	CAGACUAUGCAGAUGGGAGUGAA	1463		APP:811L21 antisense siNA (793C)	CACUCCCAUCUGCAUAGUCTT	1503
829	GUAGCAGAGGAGGAAGAAGUGGC	1464		APP:849L21 antisense siNA (831C)	CACUUCUUCCUCCUCUGCUTT	1504
851	CUGAGGUGGAAGAAGAAGAAGCC	1465		APP:871L21 antisense siNA (853C)	CUUCUUCUUCUUCCACCUCTT	1505
1356	AGAGAGAAUGUCCCAGGUCAUGA	1466		APP:1376L21 antisense siNA (1358C)	AUGACCUGGGACAUUCUCUTT	1506
1568	AGAACUACAUCACCGCUCUGCAG	1467		APP:1588L21 antisense siNA (1570C)	GCAGAGCGGUGAUGUAGUUTT	1507
2012	AUUCUUUUGGGGCUGACUCUGUG	1468		APP:2032L21 antisense siNA (2014C)	CAGAGUCAGCCCCAAAAGATT	1508
2481	UGAAGUUGGACAGCAAAACCAUU	1469		APP:2501L21 antisense siNA (2483C)	UGGUUUUGCUGUCCAACUUTT	1509
2482	GAAGUUGGACAGCAAAACCAUUG	1470		APP:2502L21 antisense siNA (2484C)	AUGGUUUUGCUGUCCAACUTT	1510
791	CAGACUAUGCAGAUGGGAGUGAA	1463		APP:793U21 sense siNA stab04	B GAcuAuGcAGAuGGGAGuGTT B	1511
829	GUAGCAGAGGAGGAAGAAGUGGC	1464		APP:831U21 sense siNA stab04	B AGcAGAGGAGGAAGAAGuGTT B	1512
851	CUGAGGUGGAAGAAGAAGAAGCC	1465		APP:853U21 sense siNA stab04	B GAGGuGGAAGAAGAAGAAGTT B	1513
1356	AGAGAGAAUGUCCCAGGUCAUGA	1466		APP:1358U21 sense siNA stab04	B AGAGAAuGucccAGGucAuTT B	1514
1568	AGAACUACAUCACCGCUCUGCAG	1467		APP:1570U21 sense siNA stab04	B AAcuAcAucAccGcucuGcTT B	1515
2012	AUUCUUUUGGGGCUGACUCUGUG	1468		APP:2014U21 sense siNA stab04	B ucuuuuGGGGcuGAcucuGTT B	1516
2481	UGAAGUUGGACAGCAAAACCAUU	1469		APP:2483U21 sense siNA stab04	B AAGuuGGAcAGcAAAAccATT B	1517
2482	GAAGUUGGACAGCAAAACCAUUG	1470		APP:2484U21 sense siNA stab04	B AGuuGGAcAGcAAAAccAuTT B	1518

791	CAGACUAUGCAGAUGGGAGUGAA	1463		APP:811L21 antisense siNA (793C) stab05	cAcucccAucuGcAuAGucTsT	1519
829	GUAGCAGAGGAGGAAGAAGUGGC	1464		APP:849L21 antisense siNA (831C) stab05	cAcuucuuccucucuGcuTsT	1520
851	CUGAGGUGGAAGAAGAAGAAGCC	1465		APP:871L21 antisense siNA (853C) stab05	cuucuucuucuucuccAccucTsT	1521
1356	AGAGAGAAUGUCCCAGGUCAUGA	1466		APP:1376L21 antisense siNA (1358C) stab05	AuGAccuGGGAcAuucucuTsT	1522
1568	AGAACUACAUCACCGCUCUGCAG	1467		APP:1588L21 antisense siNA (1570C) stab05	GcAGAGcGGuGAuGuAGuuTsT	1523
2012	AUUCUUUUGGGGCUGACUCUGUG	1468		APP:2032L21 antisense siNA (2014C) stab05	cAGAGucAGccccAAAAGATsT	1524
2481	UGAAGUUGGACAGCAAAACCAUU	1469		APP:2501L21 antisense siNA (2483C) stab05	uGGuuuuGcuGuccAAcuuTsT	1525
2482	GAAGUUGGACAGCAAAACCAUUG	1470		APP:2502L21 antisense siNA (2484C) stab05	AuGGuuuuGcuGuccAAcuTsT	1526
791	CAGACUAUGCAGAUGGGAGUGAA	1463		APP:793U21 sense siNA stab07	B *GAcuAuGcAGAuGGGAGUGTT* B	1527
829	GUAGCAGAGGAGGAAGAAGUGGC	1464		APP:831U21 sense siNA stab07	B *AGcAGAGGAGGAAGAAGUGTT* B	1528
851	CUGAGGUGGAAGAAGAAGAAGCC	1465		APP:853U21 sense siNA stab07	B *GAGGuGGAAGAAGAAGAAGTT* B	1529
1356	AGAGAGAAUGUCCCAGGUCAUGA	1466		APP:1358U21 sense siNA stab07	B *AGAGAAuGucccAGGucAuTT* B	1530
1568	AGAACUACAUCACCGCUCUGCAG	1467		APP:1570U21 sense siNA stab07	B *AAcuAcAucAccGcucuGcTT* B	1531
2012	AUUCUUUUGGGGCUGACUCUGUG	1468		APP:2014U21 sense siNA stab07	B ucuuuuGGGGcuGAcucuGTT B	1532
2481	UGAAGUUGGACAGCAAAACCAUU	1469		APP:2483U21 sense siNA stab07	B *AAGuuGGAcAGcAAAAccATT* B	1533
2482	GAAGUUGGACAGCAAAACCAUUG	1470		APP:2484U21 sense siNA stab07	B *AGuuGGAcAGcAAAAccAuTT* B	1534
791	CAGACUAUGCAGAUGGGAGUGAA	1463		APP:811L21 antisense siNA (793C) stab11	*cAcucccAucuGcAuAGucTsT*	1535
829	GUAGCAGAGGAGGAAGAAGUGGC	1464		APP:849L21 antisense siNA (831C) stab11	*cAcuucuuccucucuGcuTsT*	1536
851	CUGAGGUGGAAGAAGAAGAAGCC	1465		APP:871L21 antisense siNA (853C) stab11	*cuucuucuucuucuccAccucTsT*	1537
1356	AGAGAGAAUGUCCCAGGUCAUGA	1466		APP:1376L21 antisense siNA (1358C) stab11	*AuGAccuGGGAcAuucucuTsT*	1538
1568	AGAACUACAUCACCGCUCUGCAG	1467		APP:1588L21 antisense siNA (1570C) stab11	*GcAGAGcGGuGAuGuAGuuTsT*	1539
2012	AUUCUUUUGGGGCUGACUCUGUG	1468		APP:2032L21 antisense siNA (2014C) stab11	*cAGAGucAGccccAAAAGATsT*	1540
2481	UGAAGUUGGACAGCAAAACCAUU	1469		APP:2501L21 antisense siNA (2483C) stab11	*uGGuuuuGcuGuccAAcuuTsT*	1541

2482	GAAGUUGGACAGCAAAACCAUUG	1470		APP:2502L21 antisense siNA (2484C) stab11	AuGGuuuuGcuGuccAAcuTsT	1542
791	CAGACUAUGCAGAUGGGAGUGAA	1463		APP:793U21 sense siNA stab18	B GAcuAuGcAGAuGGGAGuGTT B	1543
829	GUAGCAGAGGAGGAAGAAGUGGC	1464		APP:831U21 sense siNA stab18	B AGcAGAGGAGGAAGAAGuGTT B	1544
851	CUGAGGUGGAAGAAGAAGAAGCC	1465		APP:853U21 sense siNA stab18	B GAGGuGGAAGAAGAAGAAGTT B	1545
1356	AGAGAGAAUGUCCCAGGUCAUGA	1466		APP:1358U21 sense siNA stab18	B AGAGAAuGucccAGGucAuTT B	1546
1568	AGAACUACAUCACCGCUCUGCAG	1467		APP:1570U21 sense siNA stab18	B AAcuAcAucAccGcucuGcTT B	1547
2012	AUUCUUUUGGGGCUGACUCUGUG	1468		APP:2014U21 sense siNA stab18	B ucuuuuGGGGcuGAcucuGTT B	1548
2481	UGAAGUUGGACAGCAAAACCAUU	1469		APP:2483U21 sense siNA stab18	B AAGuuGGGAcAGcAAAAccATT B	1549
2482	GAAGUUGGACAGCAAAACCAUUG	1470		APP:2484U21 sense siNA stab18	B AGuuGGAcAGcAAAAccAuTT B	1550
791	CAGACUAUGCAGAUGGGAGUGAA	1463	33885	APP:811L21 antisense siNA (793C) stab08	cAcucccAucuGcAuAGucTsT	1551
829	GUAGCAGAGGAGGAAGAAGUGGC	1464	33886	APP:849L21 antisense siNA (831C) stab08	cAcuucuucccucccucuGcuTsT	1552
851	CUGAGGUGGAAGAAGAAGAAGCC	1465	33887	APP:871L21 antisense siNA (853C) stab08	cuucuucuucuucccAccucTsT	1553
1356	AGAGAGAAUGUCCCAGGUCAUGA	1466	33888	APP:1376L21 antisense siNA (1358C) stab08	AuGAccuGGGAcAuucucuTsT	1554
1568	AGAACUACAUCACCGCUCUGCAG	1467	33889	APP:1588L21 antisense siNA (1570C) stab08	GcAGAGcGGuGAuGuAGuuTsT	1555
2012	AUUCUUUUGGGGCUGACUCUGUG	1468	33890	APP:2032L21 antisense siNA (2014C) stab08	cAGAGucAGccccAAAAGATsT	1556
2481	UGAAGUUGGACAGCAAAACCAUU	1469	33891	APP:2501L21 antisense siNA (2483C) stab08	uGGuuuuGcuGuccAAcuuTsT	1557
2482	GAAGUUGGACAGCAAAACCAUUG	1470	33892	APP:2502L21 antisense siNA (2484C) stab08	AuGGuuuuGcuGuccAAcuTsT	1558
791	CAGACUAUGCAGAUGGGAGUGAA	1463	33869	APP:793U21 sense siNA stab09	B GACUAUGCAGAUGGGAGUGTT B	1559
829	GUAGCAGAGGAGGAAGAAGUGGC	1464	33870	APP:831U21 sense siNA stab09	B AGCAGAGGAGGAAGAAGUGTT B	1560
851	CUGAGGUGGAAGAAGAAGAAGCC	1465	33871	APP:853U21 sense siNA stab09	B GAGGUGGAAGAAGAAGAAGTT B	1561
1356	AGAGAGAAUGUCCCAGGUCAUGA	1466	33872	APP:1358U21 sense siNA stab09	B AGAGAAUGUCCCAGGUCAUTT B	1562
1568	AGAACUACAUCACCGCUCUGCAG	1467	33873	APP:1570U21 sense siNA stab09	B AACUACAUCACCGCUCUGCTT B	1563
2012	AUUCUUUUGGGGCUGACUCUGUG	1468	33874	APP:2014U21 sense siNA stab09	B UCUUUUGGGGCUGACUCUGTT B	1564
2481	UGAAGUUGGACAGCAAAACCAUU	1469	33875	APP:2483U21 sense siNA stab09	B AAGUUGGACAGCAAAACCATT B	1565
2482	GAAGUUGGACAGCAAAACCAUUG	1470	33876	APP:2484U21 sense siNA stab09	B AGUUGGACAGCAAAACCAUTT B	1566
791	CAGACUAUGCAGAUGGGAGUGAA	1463	33877	APP:811L21 antisense siNA (793C) stab10	CACUCCCAUCUGCAUAGUCTsT	1567

829	GUAGCAGAGGAGGAAGAAGUGGC	1464	33878	APP:849L21 antisense siNA (831C) stab10	CACUUCUUCCUCCUCUGCUTsT	1568
851	CUGAGGUGGAAGAAGAAGAAGCC	1465	33879	APP:871L21 antisense siNA (853C) stab10	CUUCUUCUUCUUCCACCUCTsT	1569
1356	AGAGAGAAUGUCCCAGGUCAUGA	1466	33880	APP:1376L21 antisense siNA (1358C) stab10	AUGACCUGGGACAUUCUCUTsT	1570
1568	AGAACUACAUCACCGCUCUGCAG	1467	33881	APP:1588L21 antisense siNA (1570C) stab10	GCAGAGCGGUGAUGUAGUUTsT	1571
2012	AUUCUUUUGGGGCUGACUCUGUG	1468	33882	APP:2032L21 antisense siNA (2014C) stab10	CAGAGUCAGCCCCAAAAGATsT	1572
2481	UGAAGUUGGACAGCAAAACCAUU	1469	33883	APP:2501L21 antisense siNA (2483C) stab10	UGGUUUUGCUGUCCAACUUTsT	1573
2482	GAAGUUGGACAGCAAAACCAUUG	1470	33884	APP:2502L21 antisense siNA (2484C) stab10	AUGGUUUUGCUGUCCAACUTsT	1574
791	CAGACUAUGCAGAUGGGAGUGAA	1463		APP:811L21 antisense siNA (793C) stab19	cAcucccAucuGcAuAGucTT B	1575
829	GUAGCAGAGGAGGAAGAAGUGGC	1464		APP:849L21 antisense siNA (831C) stab19	cAcuucuucccucccucuGcuTT B	1576
851	CUGAGGUGGAAGAAGAAGAAGCC	1465		APP:871L21 antisense siNA (853C) stab19	cuucuucuucuucccAccucTT B	1577
1356	AGAGAGAAUGUCCCAGGUCAUGA	1466		APP:1376L21 antisense siNA (1358C) stab19	AuGAccuGGGAcAuucucuTT B	1578
1568	AGAACUACAUCACCGCUCUGCAG	1467		APP:1588L21 antisense siNA (1570C) stab19	GcAGAGcGGuGAuGuAGuuTT B	1579
2012	AUUCUUUUGGGGCUGACUCUGUG	1468		APP:2032L21 antisense siNA (2014C) stab19	cAGAGucAGccccAAAAGATT B	1580
2481	UGAAGUUGGACAGCAAAACCAUU	1469		APP:2501L21 antisense siNA (2483C) stab19	uGGuuuuGcuGuccAAcuuTT B	1581
2482	GAAGUUGGACAGCAAAACCAUUG	1470		APP:2502L21 antisense siNA (2484C) stab19	AuGGuuuuGcuGuccAAcuTT B	1582
791	CAGACUAUGCAGAUGGGAGUGAA	1463		APP:811L21 antisense siNA (793C) stab22	CACUCCCAUCUGCAUAGUCTT B	1583
829	GUAGCAGAGGAGGAAGAAGUGGC	1464		APP:849L21 antisense siNA (831C) stab22	CACUUCUUCCUCCUCUGCUTT B	1584
851	CUGAGGUGGAAGAAGAAGAAGCC	1465		APP:871L21 antisense siNA (853C) stab22	CUUCUUCUUCUUCCACCUCTT B	1585
1356	AGAGAGAAUGUCCCAGGUCAUGA	1466		APP:1376L21 antisense siNA (1358C) stab22	AUGACCUGGGACAUUCUCUTT B	1586
1568	AGAACUACAUCACCGCUCUGCAG	1467		APP:1588L21 antisense siNA (1570C) stab22	GCAGAGCGGUGAUGUAGUUTT B	1587

2012	AUUCUUUUGGGGCUGACUCUGUG	1468		APP:2032L21 antisense siNA (2014C) stab22	CAGAGUCAGCCCCAAAAGATT B	1588
2481	UGAAGUUGGACAGCAAAACCAUU	1469		APP:2501L21 antisense siNA (2483C) stab22	UGGUUUUGCUGUCCAACUUTT B	1589
2482	GAAGUUGGACAGCAAAACCAUUG	1470		APP:2502L21 antisense siNA (2484C) stab22	AUGGUUUUGCUGUCCAACUTT B	1590

BACE

Target Pos	Target	Seq ID	Cmpd#	Aliases	Sequence	Seq ID
1025	CCUGGAGCCUUUCUUUGACUCUC	1471		BACE:1027U21 sense siNA	UGGAGCCUUUCUUUGACUCTT	1591
1028	GGAGCCUUUCUUUGACUCUCUGG	1472		BACE:1030U21 sense siNA	AGCCUUUCUUUGACUCUCUTT	1592
1393	AGAAGUUCCCUGAUGGUUUCUGG	1473		BACE:1395U21 sense siNA	AAGUUCCCUGAUGGUUUCUTT	1593
1490	AAUGGGUGAGGUUACCAACCAGU	1474	31005	BACE:1492U21 sense siNA	UGGGUGAGGUUACCAACCATT	1594
1753	UCACCUUGGACAUGGAAGACUGU	1475	31006	BACE:1755U21 sense siNA	ACCUUGGACAUGGAAGACUTT	1595
1803	UCAACCCUCAUGACCAUAGCCUA	1476		BACE:1805U21 sense siNA	AACCCUCAUGACCAUAGCCTT	1596
2457	CCUAACAUUGGUGCAAAGAUUGC	1477	31007	BACE:2459U21 sense siNA	UAACAUUGGUGCAAAGAUUTT	1597
3583	UAUGGGACCUGCUAAGUGUGGAA	1478	31008	BACE:3585U21 sense siNA	UGGGACCUGCUAAGUGUGGTT	1598
1025	CCUGGAGCCUUUCUUUGACUCUC	1471		BACE:1045L21 antisense siNA (1027C)	GAGUCAAAGAAAGGCUCCATT	1599
1028	GGAGCCUUUCUUUGACUCUCUGG	1472		BACE:1048L21 antisense siNA (1030C)	AGAGAGUCAAAGAAAGGCUTT	1600
1393	AGAAGUUCCCUGAUGGUUUCUGG	1473		BACE:1413L21 antisense siNA (1395C)	AGAAACCAUCAGGGAACUUTT	1601
1490	AAUGGGUGAGGUUACCAACCAGU	1474	31081	BACE:1510L21 antisense siNA (1492C)	UGGUUGGUAACCUCACCCATT	1602
1753	UCACCUUGGACAUGGAAGACUGU	1475	31082	BACE:1773L21 antisense siNA (1755C)	AGUCUUCCAUGUCCAAGGUTT	1603
1803	UCAACCCUCAUGACCAUAGCCUA	1476		BACE:1823L21 antisense siNA (1805C)	GGCUAUGGUCAUGAGGGUUTT	1604
2457	CCUAACAUUGGUGCAAAGAUUGC	1477	31083	BACE:2477L21 antisense siNA (2459C)	AAUCUUUGCACCAAUGUUATT	1605
3583	UAUGGGACCUGCUAAGUGUGGAA	1478	31084	BACE:3603L21 antisense siNA (3585C)	CCACACUUAGCAGGUCCCATT	1606
1025	CCUGGAGCCUUUCUUUGACUCUC	1471		BACE:1027U21 sense siNA stab04	B uGGAGCcuuucuuuGAcucTT B	1607
1028	GGAGCCUUUCUUUGACUCUCUGG	1472		BACE:1030U21 sense siNA stab04	B AGccuuucuuuGAcucucuTT B	1608
1393	AGAAGUUCCCUGAUGGUUUCUGG	1473		BACE:1395U21 sense siNA stab04	B AAGuucccuGAuGGuuucuTT B	1609
1490	AAUGGGUGAGGUUACCAACCAGU	1474	30729	BACE:1492U21 sense siNA stab04	B uGGGuGAGGuuuAccAAccATT B	1610
1753	UCACCUUGGACAUGGAAGACUGU	1475	30730	BACE:1755U21 sense siNA stab04	B AccuuGGAcAuGGAAGAcuTT B	1611
1803	UCAACCCUCAUGACCAUAGCCUA	1476		BACE:1805U21 sense siNA stab04	B AAcccucAuGAccAuAGccTT B	1612
2457	CCUAACAUUGGUGCAAAGAUUGC	1477	31378	BACE:2459U21 sense siNA stab04	B uAAcAuuGGuGcAAAGAuuTT B	1613
3583	UAUGGGACCUGCUAAGUGUGGAA	1478	30732	BACE:3585U21 sense siNA stab04	B uGGGAccuGcuAAGuGuGGTT B	1614

1025	CCUGGAGCCUUUCUUUGACUCUC	1471		BACE:1045L21 antisense siNA (1027C) stab05	GAGucAAAGAAAGGcuccATsT	1615
1028	GGAGCCUUUCUUUGACUCUCUGG	1472		BACE:1048L21 antisense siNA (1030C) stab05	AGAGAGucAAAGAAAGGcuTsT	1616
1393	AGAAGUUCCCUGAUGGUUUCUGG	1473		BACE:1413L21 antisense siNA (1395C) stab05	AGAAAccAucAGGGAAcuuTsT	1617
1490	AAUGGGUGAGGUUACCAACCAGU	1474	30733	BACE:1510L21 antisense siNA (1492C) stab05	uGGuuGGuAAccucAcccATsT	1618
1753	UCACCUUGGACAUGGAAGACUGU	1475	30734	BACE:1773L21 antisense siNA (1755C) stab05	AGucuucAuGuccAAGGuTsT	1619
1803	UCAACCCUCAUGACCAUAGCCUA	1476		BACE:1823L21 antisense siNA (1805C) stab05	GGcuAuGGucAuGAGGGuuTsT	1620
2457	CCUAACAUUGGUGCAAAGAUUGC	1477	31381	BACE:2477L21 antisense siNA (2459C) stab05	AAucuuuGcAccAAuGuuATsT	1621
3583	UAUGGGACCUGCUAAGUGUGGAA	1478	30736	BACE:3603L21 antisense siNA (3585C) stab05	ccAcAcuuAGcAGGucccATsT	1622
1025	CCUGGAGCCUUUCUUUGACUCUC	1471		BACE:1027U21 sense siNA stab07	B u*GGAGCcuuucuuuGAcucTT* B	1623
1028	GGAGCCUUUCUUUGACUCUCUGG	1472		BACE:1030U21 sense siNA stab07	B *AGccuuucuuuGAcucucuTT* B	1624
1393	AGAAGUUCCCUGAUGGUUUCUGG	1473		BACE:1395U21 sense siNA stab07	B *AAGuucccuGAuGGuuucuTT* B	1625
1490	AAUGGGUGAGGUUACCAACCAGU	1474		BACE:1492U21 sense siNA stab07	B u*GGGuGAGGuuAccAAccATT* B	1626
1753	UCACCUUGGACAUGGAAGACUGU	1475		BACE:1755U21 sense siNA stab07	B *AccuuGGAcAuGGAAGAcuTT* B	1627
1803	UCAACCCUCAUGACCAUAGCCUA	1476		BACE:1805U21 sense siNA stab07	B *AAcccucAuGAccAuAGccTT* B	1628
2457	CCUAACAUUGGUGCAAAGAUUGC	1477	31384	BACE:2459U21 sense siNA stab07	B u*AAcAuuGGuGcAAAGAuuTT* B	1629
3583	UAUGGGACCUGCUAAGUGUGGAA	1478		BACE:3585U21 sense siNA stab07	B u*GGGAccuGcuAAGuGuGGTT* B	1630
1025	CCUGGAGCCUUUCUUUGACUCUC	1471		BACE:1045L21 antisense siNA (1027C) stab11	*GAGucAAAGAAAGGcuccATsT*	1631
1028	GGAGCCUUUCUUUGACUCUCUGG	1472		BACE:1048L21 antisense siNA (1030C) stab11	*AGAGAGucAAAGAAAGGcuTsT*	1632
1393	AGAAGUUCCCUGAUGGUUUCUGG	1473		BACE:1413L21 antisense siNA (1395C) stab11	*AGAAAccAucAGGGAAcuuTsT*	1633
1490	AAUGGGUGAGGUUACCAACCAGU	1474		BACE:1510L21 antisense siNA (1492C) stab11	*uGGuuGGuAAccucAcccATsT*	1634
1753	UCACCUUGGACAUGGAAGACUGU	1475		BACE:1773L21 antisense siNA (1755C) stab11	*AGucuucAuGuccAAGGuTsT*	1635
1803	UCAACCCUCAUGACCAUAGCCUA	1476		BACE:1823L21 antisense siNA (1805C) stab11	*GGcuAuGGucAuGAGGGuuTsT*	1636
2457	CCUAACAUUGGUGCAAAGAUUGC	1477	31387	BACE:2477L21 antisense siNA (2459C) stab11	*AAucuuuGcAccAAuGuuATsT*	1637

3583	UAUGGGACCUGCUAAGUGUGGAA	1478		BACE:3603L21 antisense siNA (3585C) stab11	ccAcAcuuAGcAGGucccATsT	1638
1025	CCUGGAGCCUUUCUUUGACUCUC	1471		BACE:1027U21 sense siNA stab18	B uGGAGccuuucuuuGAcucTT B	1639
1028	GGAGCCUUUCUUUGACUCUCUGG	1472		BACE:1030U21 sense siNA stab18	B AGccuuucuuuGAcucucuTT B	1640
1393	AGAAGUUCCCUGAUGGUUUCUGG	1473		BACE:1395U21 sense siNA stab18	B AAGuucccuGAuGGuuucuTT B	1641
1490	AAUGGGUGAGGUUACCAACCAGU	1474		BACE:1492U21 sense siNA stab18	B uGGGuGAGGuuAccAAccATT B	1642
1753	UCACCUUGGACAUGGAAGACUGU	1475		BACE:1755U21 sense siNA stab18	B AccuuGGAcAuGGAAGAcuTT B	1643
1803	UCAACCCUCAUGACCAUAGCCUA	1476		BACE:1805U21 sense siNA stab18	B AAcccucAuGAccAuAGccTT B	1644
2457	CCUAACAUUGGUGCAAAGAUUGC	1477		BACE:2459U21 sense siNA stab18	B uAAcAuuGGuGcAAAGAuuTT B	1645
3583	UAUGGGACCUGCUAAGUGUGGAA	1478		BACE:3585U21 sense siNA stab18	B uGGGAccuGcuAAGuGuGGTT B	1646
1025	CCUGGAGCCUUUCUUUGACUCUC	1471		BACE:1045L21 antisense siNA (1027C) stab08	GAGucAAAGAAAGGcuccATsT	1647
1028	GGAGCCUUUCUUUGACUCUCUGG	1472		BACE:1048L21 antisense siNA (1030C) stab08	AGAGAGucAAAGAAAGGcuTsT	1648
1393	AGAAGUUCCCUGAUGGUUUCUGG	1473		BACE:1413L21 antisense siNA (1395C) stab08	AGAAAccAucAGGGAAcuuTsT	1649
1490	AAUGGGUGAGGUUACCAACCAGU	1474		BACE:1510L21 antisense siNA (1492C) stab08	uGGuuGGuAAccucAcccATsT	1650
1753	UCACCUUGGACAUGGAAGACUGU	1475		BACE:1773L21 antisense siNA (1755C) stab08	AGucuuccAuGuccAAGGuTsT	1651
1803	UCAACCCUCAUGACCAUAGCCUA	1476		BACE:1823L21 antisense siNA (1805C) stab08	GGcuAuGGucAuGAGGGuuTsT	1652
2457	CCUAACAUUGGUGCAAAGAUUGC	1477		BACE:2477L21 antisense siNA (2459C) stab08	AAucuuuGcAccAAuGuuATsT	1653
3583	UAUGGGACCUGCUAAGUGUGGAA	1478		BACE:3603L21 antisense siNA (3585C) stab08	ccAcAcuuAGcAGGucccATsT	1654
1025	CCUGGAGCCUUUCUUUGACUCUC	1471		BACE:1027U21 sense siNA stab09	B UGGAGCCUUUCUUUGACUCTT B	1655
1028	GGAGCCUUUCUUUGACUCUCUGG	1472		BACE:1030U21 sense siNA stab09	B AGCCUUUCUUUGACUCUCUTT B	1656
1393	AGAAGUUCCCUGAUGGUUUCUGG	1473		BACE:1395U21 sense siNA stab09	B AAGUUCCCUGAUGGUUUCUTT B	1657
1490	AAUGGGUGAGGUUACCAACCAGU	1474		BACE:1492U21 sense siNA stab09	B UGGGUGAGGUUACCAACCATT B	1658
1753	UCACCUUGGACAUGGAAGACUGU	1475		BACE:1755U21 sense siNA stab09	B ACCUUGGACAUGGAAGACUTT B	1659
1803	UCAACCCUCAUGACCAUAGCCUA	1476		BACE:1805U21 sense siNA stab09	B AACCCUCAUGACCAUAGCCTT B	1660
2457	CCUAACAUUGGUGCAAAGAUUGC	1477		BACE:2459U21 sense siNA stab09	B UAACAUUGGUGCAAAGAUUTT B	1661
3583	UAUGGGACCUGCUAAGUGUGGAA	1478		BACE:3585U21 sense siNA stab09	B UGGGACCUGCUAAGUGUGGTT B	1662

1025	CCUGGAGCCUUUCUUUGACUCUC	1471		BACE:1045L21 antisense siNA (1027C) stab10	GAGUCAAAGAAAGGCUCCATsT	1663
1028	GGAGCCUUUCUUUGACUCUCUGG	1472		BACE:1048L21 antisense siNA (1030C) stab10	AGAGAGUCAAAGAAAGGCUTsT	1664
1393	AGAAGUUCCCUGAUGGUUUCUGG	1473		BACE:1413L21 antisense siNA (1395C) stab10	AGAAACCAUCAGGGAACUUTsT	1665
1490	AAUGGGUGAGGUUACCAACCAGU	1474		BACE:1510L21 antisense siNA (1492C) stab10	UGGUUGGUAACCUCACCCATsT	1666
1753	UCACCUUGGACAUGGAAGACUGU	1475		BACE:1773L21 antisense siNA (1755C) stab10	AGUCUUCCAUGUCCAAGGUTsT	1667
1803	UCAACCCUCAUGACCAUAGCCUA	1476		BACE:1823L21 antisense siNA (1805C) stab10	GGCUAUGGUCAUGAGGGUUTsT	1668
2457	CCUAACAUUGGUGCAAAGAUUGC	1477		BACE:2477L21 antisense siNA (2459C) stab10	AAUCUUUGCACCAAUGUUATsT	1669
3583	UAUGGGACCUGCUAAGUGUGGAA	1478		BACE:3603L21 antisense siNA (3585C) stab10	CCACACUUAGCAGGUCCCATsT	1670
1025	CCUGGAGCCUUUCUUUGACUCUC	1471		BACE:1045L21 antisense siNA (1027C) stab19	GAGucAAAGAAAGGcuccATT B	1671
1028	GGAGCCUUUCUUUGACUCUCUGG	1472		BACE:1048L21 antisense siNA (1030C) stab19	AGAGAGucAAAGAAAGGcuTT B	1672
1393	AGAAGUUCCCUGAUGGUUUCUGG	1473		BACE:1413L21 antisense siNA (1395C) stab19	AGAAAccAucAGGGAAcuuTT B	1673
1490	AAUGGGUGAGGUUACCAACCAGU	1474		BACE:1510L21 antisense siNA (1492C) stab19	uGGuuGGuAAccucAcccATT B	1674
1753	UCACCUUGGACAUGGAAGACUGU	1475		BACE:1773L21 antisense siNA (1755C) stab19	AGucuuccAuGuccAAGGuTT B	1675
1803	UCAACCCUCAUGACCAUAGCCUA	1476		BACE:1823L21 antisense siNA (1805C) stab19	GGcuAuGGucAuGAGGGuuTT B	1676
2457	CCUAACAUUGGUGCAAAGAUUGC	1477		BACE:2477L21 antisense siNA (2459C) stab19	AAucuuuGcAccAAuGuuATT B	1677
3583	UAUGGGACCUGCUAAGUGUGGAA	1478		BACE:3603L21 antisense siNA (3585C) stab19	ccAcAcuuAGcAGGucccATT B	1678
1025	CCUGGAGCCUUUCUUUGACUCUC	1471		BACE:1045L21 antisense siNA (1027C) stab22	GAGUCAAAGAAAGGCUCCATT B	1679
1028	GGAGCCUUUCUUUGACUCUCUGG	1472		BACE:1048L21 antisense siNA (1030C) stab22	AGAGAGUCAAAGAAAGGCUTT B	1680
1393	AGAAGUUCCCUGAUGGUUUCUGG	1473		BACE:1413L21 antisense siNA (1395C) stab22	AGAAACCAUCAGGGAACUUTT B	1681
1490	AAUGGGUGAGGUUACCAACCAGU	1474		BACE:1510L21 antisense siNA (1492C) stab22	UGGUUGGUAACCUCACCCATT B	1682

1753	UCACCUUGGACAUGGAAGACUGU	1475		BACE:1773L21 antisense siNA (1755C) stab22	AGUCUUCCAUGUCCAAGGUTT B	1683
1803	UCAACCCUCAUGACCAUAGCCUA	1476		BACE:1823L21 antisense siNA (1805C) stab22	GGCUAUGGUCAUGAGGGUUTT B	1684
2457	CCUAACAUUGGUGCAAAGAUUGC	1477		BACE:2477L21 antisense siNA (2459C) stab22	AAUCUUUGCACCAAUGUUATT B	1685
3583	UAUGGGACCUGCUAAGUGUGGAA	1478		BACE:3603L21 antisense siNA (3585C) stab22	CCACACUUAGCAGGUCCCATT B	1686
2457	CCUAACAUUGGUGCAAAGAUUGC	657	31390	BACE:2459U21 sense siNA inv stab04	B uuAGAAAcGuGGuuAcAAuTT B	1687
2457	CCUAACAUUGGUGCAAAGAUUGC	657	31393	BACE:2477L21 antisense siNA (2459C) inv stab05	AuuGuAAccAcGuuucuAATsT	1688
2457	CCUAACAUUGGUGCAAAGAUUGC	657	31396	BACE:2459U21 sense siNA inv stab07	B uu*AGAAAcGuGGuuAcAAu*TT B	1689
2457	CCUAACAUUGGUGCAAAGAUUGC	657	31399	BACE:2477L21 antisense siNA (2459C) inv stab11	*AuuGuAAccAcGuuucuAA*TsT	1690

PSEN1

Target Pos	Target	Seq ID	Cmpd#	Aliases	Sequence	Seq ID
693	CUAAUGGACGACCCCAGGGUAAC	1479		PSEN1:695U21 sense siNA	AAUGGACGACCCCAGGGUATT	1691
1131	CUGUUGCACUCCUGAUCUGGAAU	1480		PSEN1:1133U21 sense siNA	GUUGCACUCCUGAUCUGGATT	1692
1493	GAAAGCACAGAAAGGGAGUCACA	1481		PSEN1:1495U21 sense siNA	AAGCACAGAAAGGGAGUCATT	1693
1505	AGGGAGUCACAAGACACUGUUGC	1482		PSEN1:1507U21 sense siNA	GGAGUCACAAGACACUGUUTT	1694
1748	GACUGGAACACAACCAUAGCCUG	1483		PSEN1:1750U21 sense siNA	CUGGAACACAACCAUAGCCTT	1695
1751	UGGAACACAACCAUAGCCUGUUU	1484		PSEN1:1753U21 sense siNA	GAACACAACCAUAGCCUGUTT	1696
2184	CUACCAGAUUUGAGGGACGAGGU	1485		PSEN1:2186U21 sense siNA	ACCAGAUUUGAGGGACGAGTT	1697
3007	UGUAUGCCCAAAGCGGUAGAAUU	1486		PSEN1:3009U21 sense siNA	UAUGCCCAAAGCGGUAGAATT	1698
693	CUAAUGGACGACCCCAGGGUAAC	1479		PSEN1:713L21 antisense siNA (695C)	UACCCUGGGGUCCGUCCAUUTT	1699
1131	CUGUUGCACUCCUGAUCUGGAAU	1480		PSEN1:1151L21 antisense siNA (1133C)	UCCAGAUCAGGAGUGCAACTT	1700
1493	GAAAGCACAGAAAGGGAGUCACA	1481		PSEN1:1513L21 antisense siNA (1495C)	UGACUCCCUUUCUGUGCUUTT	1701
1505	AGGGAGUCACAAGACACUGUUGC	1482		PSEN1:1525L21 antisense siNA (1507C)	AACAGUGUCUUGUGACUCCTT	1702
1748	GACUGGAACACAACCAUAGCCUG	1483		PSEN1:1768L21 antisense siNA (1750C)	GGCUAUGGUUGUGUUCCAGTT	1703
1751	UGGAACACAACCAUAGCCUGUUU	1484		PSEN1:1771L21 antisense siNA (1753C)	ACAGGCUAUGGUUGUGUUCTT	1704
2184	CUACCAGAUUUGAGGGACGAGGU	1485		PSEN1:2204L21 antisense siNA (2186C)	CUCGUCCCUCAAAUCUGGUTT	1705
3007	UGUAUGCCCAAAGCGGUAGAAUU	1486		PSEN1:3027L21 antisense siNA (3009C)	UUCUACCGCUUUGGGCAUATT	1706
693	CUAAUGGACGACCCCAGGGUAAC	1479		PSEN1:695U21 sense siNA stab04	B AAuGGAcGAccccAGGGuATT B	1707
1131	CUGUUGCACUCCUGAUCUGGAAU	1480		PSEN1:1133U21 sense siNA stab04	B GuuGcAcuccuGAucuGGATT B	1708
1493	GAAAGCACAGAAAGGGAGUCACA	1481		PSEN1:1495U21 sense siNA stab04	B AAGcAcAGAAAGGGAGucATT B	1709
1505	AGGGAGUCACAAGACACUGUUGC	1482		PSEN1:1507U21 sense siNA stab04	B GGAGucAcAAGAcAcuGuuTT B	1710
1748	GACUGGAACACAACCAUAGCCUG	1483		PSEN1:1750U21 sense siNA stab04	B cuGGAAcAcAAccAuAGccTT B	1711

1751	UGGAACACAACCAUAGCCUGUUU	1484		PSEN1:1753U21 sense siNA stab04	B GAAcAcAAccAuAGccuGuTT B	1712
2184	CUACCAGAUUUGAGGGACGAGGU	1485		PSEN1:2186U21 sense siNA stab04	B AccAGAuuuGAGGGAcGAGTT B	1713
3007	UGUAUGCCCAAAGCGGUAGAAUU	1486		PSEN1:3009U21 sense siNA stab04	B uAuGcccAAAGcGGuAGAATT B	1714
693	CUAAUGGACGACCCCAGGGUAAC	1479		PSEN1:713L21 antisense siNA (695C) stab05	uAcccuGGGGucGuccAuuTsT	1715
1131	CUGUUGCACUCCUGAUCUGGAAU	1480		PSEN1:1151L21 antisense siNA (1133C) stab05	uccAGAucAGGAGuGcAAcTsT	1716
1493	GAAAGCACAGAAAGGGAGUCACA	1481		PSEN1:1513L21 antisense siNA (1495C) stab05	uGAcucccuuucuGuGcuuTsT	1717
1505	AGGGAGUCACAAGACACUGUUGC	1482		PSEN1:1525L21 antisense siNA (1507C) stab05	AAcAGuGucuuGuGAcuccTsT	1718
1748	GACUGGAACACAACCAUAGCCUG	1483		PSEN1:1768L21 antisense siNA (1750C) stab05	GGcuAuGGuuGuGuuccAGTsT	1719
1751	UGGAACACAACCAUAGCCUGUUU	1484		PSEN1:1771L21 antisense siNA (1753C) stab05	AcAGGcuAuGGuuGuGuucTsT	1720
2184	CUACCAGAUUUGAGGGACGAGGU	1485		PSEN1:2204L21 antisense siNA (2186C) stab05	cucGucccucAAAucuGGuTsT	1721
3007	UGUAUGCCCAAAGCGGUAGAAUU	1486		PSEN1:3027L21 antisense siNA (3009C) stab05	uucuAccGcuuuGGGcAuATsT	1722
693	CUAAUGGACGACCCCAGGGUAAC	1479		PSEN1:695U21 sense siNA stab07	B *AAuGGAcGAcccAGGGuATT* B	1723
1131	CUGUUGCACUCCUGAUCUGGAAU	1480		PSEN1:1133U21 sense siNA stab07	B *GuuGcAcuccuGAucuGGATT* B	1724
1493	GAAAGCACAGAAAGGGAGUCACA	1481		PSEN1:1495U21 sense siNA stab07	B *AAGcAcAGAAAGGGAGucATT* B	1725
1505	AGGGAGUCACAAGACACUGUUGC	1482		PSEN1:1507U21 sense siNA stab07	B *GGAGucAcAAGAcAcuGuuTT* B	1726
1748	GACUGGAACACAACCAUAGCCUG	1483		PSEN1:1750U21 sense siNA stab07	B *cuGGAAcAcAAccAuAGccTT* B	1727
1751	UGGAACACAACCAUAGCCUGUUU	1484		PSEN1:1753U21 sense siNA stab07	B *GAAcAcAAccAuAGccuGuTT* B	1728
2184	CUACCAGAUUUGAGGGACGAGGU	1485		PSEN1:2186U21 sense siNA stab07	B *AccAGAuuuGAGGGAcGAGTT* B	1729
3007	UGUAUGCCCAAAGCGGUAGAAUU	1486		PSEN1:3009U21 sense siNA stab07	B *uAuGcccAAAGcGGuAGAATT* B	1730
693	CUAAUGGACGACCCCAGGGUAAC	1479		PSEN1:713L21 antisense siNA (695C) stab11	*uAcccuGGGGucGuccAuuTsT*	1731

1131	CUGUUGCACUCCUGAUCUGGAAU	1480		PSEN1:1151L21 antisense siNA (1133C) stab11	uccAGAucAGGAGuGcAAcTsT	1732
1493	GAAAGCACAGAAAGGGAGUCACA	1481		PSEN1:1513L21 antisense siNA (1495C) stab11	uGAcucccuuucuGuGcuuTsT	1733
1505	AGGGAGUCACAAGACACUGUUGC	1482		PSEN1:1525L21 antisense siNA (1507C) stab11	AAcAGuGucuuGuGAcuccTsT	1734
1748	GACUGGAACACAACCAUAGCCUG	1483		PSEN1:1768L21 antisense siNA (1750C) stab11	GGcuAuGGuuGuGuuccAGTsT	1735
1751	UGGAACACAACCAUAGCCUGUUU	1484		PSEN1:1771L21 antisense siNA (1753C) stab11	AcAGGcuAuGGuuGuGuucTsT	1736
2184	CUACCAGAUUUGAGGGACGAGGU	1485		PSEN1:2204L21 antisense siNA (2186C) stab11	cucGucccucAAAucuGGuTsT	1737
3007	UGUAUGCCCAAAGCGGUAGAAUU	1486		PSEN1:3027L21 antisense siNA (3009C) stab11	uucuAccGcuuuGGGcAuATsT	1738
693	CUAAUGGACGACCCCAGGGUAAC	1479		PSEN1:695U21 sense siNA stab18	B AAuGGAcGAcccAGGGuATT B	1739
1131	CUGUUGCACUCCUGAUCUGGAAU	1480		PSEN1:1133U21 sense siNA stab18	B GuuGcAcuccuGAucuGGATT B	1740
1493	GAAAGCACAGAAAGGGAGUCACA	1481		PSEN1:1495U21 sense siNA stab18	B AAGcAcAGAAAGGGAGucATT B	1741
1505	AGGGAGUCACAAGACACUGUUGC	1482		PSEN1:1507U21 sense siNA stab18	B GGAGucAcAAGAcAcuGuuTT B	1742
1748	GACUGGAACACAACCAUAGCCUG	1483		PSEN1:1750U21 sense siNA stab18	B cuGGAAcAcAAccAuAGccTT B	1743
1751	UGGAACACAACCAUAGCCUGUUU	1484		PSEN1:1753U21 sense siNA stab18	B GAAcAcAAccAuAGccuGuTT B	1744
2184	CUACCAGAUUUGAGGGACGAGGU	1485		PSEN1:2186U21 sense siNA stab18	B AccAGAuuuGAGGGAcGAGTT B	1745
3007	UGUAUGCCCAAAGCGGUAGAAUU	1486		PSEN1:3009U21 sense siNA stab18	B uAuGcccAAAGcGGuAGAATT B	1746
693	CUAAUGGACGACCCCAGGGUAAC	1479	33933	PSEN1:713L21 antisense siNA (695C) stab08	uAcccuGGGGucGuccAuuTsT	1747
1131	CUGUUGCACUCCUGAUCUGGAAU	1480	33934	PSEN1:1151L21 antisense siNA (1133C) stab08	uccAGAucAGGAGuGcAAcTsT	1748
1493	GAAAGCACAGAAAGGGAGUCACA	1481	33935	PSEN1:1513L21 antisense siNA (1495C) stab08	uGAcucccuuucuGuGcuuTsT	1749
1505	AGGGAGUCACAAGACACUGUUGC	1482	33936	PSEN1:1525L21 antisense siNA (1507C) stab08	AAcAGuGucuuGuGAcuccTsT	1750
1748	GACUGGAACACAACCAUAGCCUG	1483	33937	PSEN1:1768L21 antisense siNA (1750C) stab08	GGcuAuGGuuGuGuuccAGTsT	1751

1751	UGGAACACAACCAUAGCCUGUUU	1484	33938	PSEN1:1771L21 antisense siNA (1753C) stab08	AcAGGcuAuGGuuGuGuucTsT	1752
2184	CUACCAGAUUUGAGGGACGAGGU	1485	33939	PSEN1:2204L21 antisense siNA (2186C) stab08	cucGucccucAAAucuGGuTsT	1753
3007	UGUAUGCCCCAAAGCGGUAGAAUU	1486	33940	PSEN1:3027L21 antisense siNA (3009C) stab08	uucuAccGcuuuGGGcAuATsT	1754
693	CUAAUGGACGACCCCAGGGUAAC	1479	33917	PSEN1:695U21 sense siNA stab09	B AAUGGACGACCCCAGGGUATT B	1755
1131	CUGUUGCACUCCUGAUCUGGAAU	1480	33918	PSEN1:1133U21 sense siNA stab09	B GUUGCACUCCUGAUCUGGATT B	1756
1493	GAAAGCACAGAAAGGGAGUCACA	1481	33919	PSEN1:1495U21 sense siNA stab09	B AAGCACAGAAAGGGAGUCATT B	1757
1505	AGGGAGUCACAAGACACUGUUGC	1482	33920	PSEN1:1507U21 sense siNA stab09	B GGAGUCACAAGACACUGUUTT B	1758
1748	GACUGGAACACAACCAUAGCCUG	1483	33921	PSEN1:1750U21 sense siNA stab09	B CUGGAACACAACCAUAGCCTT B	1759
1751	UGGAACACAACCAUAGCCUGUUU	1484	33922	PSEN1:1753U21 sense siNA stab09	B GAACACAACCAUAGCCUGUTT B	1760
2184	CUACCAGAUUUGAGGGACGAGGU	1485	33923	PSEN1:2186U21 sense siNA stab09	B ACCAGAUUUGAGGGACGAGTT B	1761
3007	UGUAUGCCCCAAAGCGGUAGAAUU	1486	33924	PSEN1:3009U21 sense siNA stab09	B UAUGCCCCAAAGCGGUAGAATT B	1762
693	CUAAUGGACGACCCCAGGGUAAC	1479	33925	PSEN1:713L21 antisense siNA (695C) stab10	UACCCUGGGGUCGUCCAUUTsT	1763
1131	CUGUUGCACUCCUGAUCUGGAAU	1480	33926	PSEN1:1151L21 antisense siNA (1133C) stab10	UCCAGAUCAGGAGUGCAACTsT	1764
1493	GAAAGCACAGAAAGGGAGUCACA	1481	33927	PSEN1:1513L21 antisense siNA (1495C) stab10	UGACUCCCUUUCUGUGCUUTsT	1765
1505	AGGGAGUCACAAGACACUGUUGC	1482	33928	PSEN1:1525L21 antisense siNA (1507C) stab10	AACAGUGUCUUGUGACUCCTsT	1766
1748	GACUGGAACACAACCAUAGCCUG	1483	33929	PSEN1:1768L21 antisense siNA (1750C) stab10	GGCUAUGGUUGUGUUCCAGTsT	1767
1751	UGGAACACAACCAUAGCCUGUUU	1484	33930	PSEN1:1771L21 antisense siNA (1753C) stab10	ACAGGCUAUGGUUGUGUUCTsT	1768
2184	CUACCAGAUUUGAGGGACGAGGU	1485	33931	PSEN1:2204L21 antisense siNA (2186C) stab10	CUCGUCCCCUCAAAUCUGGUTsT	1769
3007	UGUAUGCCCCAAAGCGGUAGAAUU	1486	33932	PSEN1:3027L21 antisense siNA (3009C) stab10	UUCUACCGCUUUGGGGCAUATsT	1770
693	CUAAUGGACGACCCCAGGGUAAC	1479		PSEN1:713L21 antisense siNA (695C) stab19	uAcccuGGGGucGuccAuuTT B	1771

1131	CUGUUGCACUCCUGAUCUGGAAU	1480		PSEN1:1151L21 antisense siNA (1133C) stab19	uccAGAucAGGAGuGcAAcTT B	1772
1493	GAAAGCACAGAAAGGGAGUCACA	1481		PSEN1:1513L21 antisense siNA (1495C) stab19	uGAcucccuuucuGuGcuuTT B	1773
1505	AGGGAGUCACAAGACACUGUUGC	1482		PSEN1:1525L21 antisense siNA (1507C) stab19	AAcAGuGucuuGuGAcuccTT B	1774
1748	GACUGGAACACAACCAUAGCCUG	1483		PSEN1:1768L21 antisense siNA (1750C) stab19	GGcuAuGGuuGuGuuccAGTT B	1775
1751	UGGAACACAACCAUAGCCUGUUU	1484		PSEN1:1771L21 antisense siNA (1753C) stab19	AcAGGcuAuGGuuGuGuucTT B	1776
2184	CUACCAGAUUUGAGGGACGAGGU	1485		PSEN1:2204L21 antisense siNA (2186C) stab19	cucGucccucAAAucuGGuTT B	1777
3007	UGUAUGCCCAAAGCGGUAGAAUU	1486		PSEN1:3027L21 antisense siNA (3009C) stab19	uucuAccGcuuuGGGcAuATT B	1778
693	CUAAUGGACGACCCCAGGGUAAC	1479		PSEN1:713L21 antisense siNA (695C) stab22	UACCCUGGGGUCGUCCAUUTT B	1779
1131	CUGUUGCACUCCUGAUCUGGAAU	1480		PSEN1:1151L21 antisense siNA (1133C) stab22	UCCAGAUCAGGAGUGCAACTT B	1780
1493	GAAAGCACAGAAAGGGAGUCACA	1481		PSEN1:1513L21 antisense siNA (1495C) stab22	UGACUCCCUUUCUGUGCUUTT B	1781
1505	AGGGAGUCACAAGACACUGUUGC	1482		PSEN1:1525L21 antisense siNA (1507C) stab22	AACAGUGUCUUGUGACUCCTT B	1782
1748	GACUGGAACACAACCAUAGCCUG	1483		PSEN1:1768L21 antisense siNA (1750C) stab22	GGCUAUGGUUGUGUUCCAGTT B	1783
1751	UGGAACACAACCAUAGCCUGUUU	1484		PSEN1:1771L21 antisense siNA (1753C) stab22	ACAGGCUAUGGUUGUGUUCTT B	1784
2184	CUACCAGAUUUGAGGGACGAGGU	1485		PSEN1:2204L21 antisense siNA (2186C) stab22	CUCGUCCCUCAAAUCUGGUTT B	1785
3007	UGUAUGCCCAAAGCGGUAGAAUU	1486		PSEN1:3027L21 antisense siNA (3009C) stab22	UUCUACCGCUUUGGGCAUATT B	1786

PSEN2

Target Pos	Target	Seq ID	Cmpd#	Aliases	Sequence	Seq ID
104	UUACUGAUGAAGAAACUGAGGCC	1487		PSEN2:106U21 sense siNA	ACUGAUGAAGAAACUGAGGTT	1787
260	AGCCAGGGAGCAUCAUUCAUUUA	1488		PSEN2:262U21 sense siNA	CCAGGGAGCAUCAUUCAUUTT	1788
549	ACCGCUAUGUCUGUAGUGGGGUU	1489		PSEN2:551U21 sense siNA	CGCUAUGUCUGUAGUGGGGTT	1789
597	AAGAGCUGACCCUCAAAUACGGA	1490		PSEN2:599U21 sense siNA	GAGCUGACCCUCAAAUACGTT	1790
730	CACGACAUUCACUGAGGACACAC	1491		PSEN2:732U21 sense siNA	CGACAUUCACUGAGGACACTT	1791
938	GUGCUCAAGACCUACAAUGUGGC	1492		PSEN2:940U21 sense siNA	GCUCAAGACCUACAAUGUGTT	1792
947	ACCUACAAUGUGGCCAUGGACUA	1493		PSEN2:949U21 sense siNA	CUACAAUGUGGCCAUGGACTT	1793
2095	GAGUGUUCCCAAUGCUUUGUCCA	1494		PSEN2:2097U21 sense siNA	GUGUUCCCAAUGCUUUGUCTT	1794
104	UUACUGAUGAAGAAACUGAGGCC	1487		PSEN2:124L21 antisense siNA (106C)	CCUCAGUUUCUUCAUCAGUTT	1795
260	AGCCAGGGAGCAUCAUUCAUUUA	1488		PSEN2:280L21 antisense siNA (262C)	AAUGAAUGAUGCUCCCUGGTT	1796
549	ACCGCUAUGUCUGUAGUGGGGUU	1489		PSEN2:569L21 antisense siNA (551C)	CCCCACUACAGACAUAGCGTT	1797
597	AAGAGCUGACCCUCAAAUACGGA	1490		PSEN2:617L21 antisense siNA (599C)	CGUAUUUGAGGGUCAGCUCTT	1798
730	CACGACAUUCACUGAGGACACAC	1491		PSEN2:750L21 antisense siNA (732C)	GUGUCCUCAGUGAAUGUCGTT	1799
938	GUGCUCAAGACCUACAAUGUGGC	1492		PSEN2:958L21 antisense siNA (940C)	CACAUUGUAGGUCUUGAGCTT	1800
947	ACCUACAAUGUGGCCAUGGACUA	1493		PSEN2:967L21 antisense siNA (949C)	GUCCAUGGCCACAUUGUAGTT	1801
2095	GAGUGUUCCCAAUGCUUUGUCCA	1494		PSEN2:2115L21 antisense siNA (2097C)	GACAAAGCAUUGGGAACACTT	1802
104	UUACUGAUGAAGAAACUGAGGCC	1487		PSEN2:106U21 sense siNA stab04	B AcuGAuGAAGAAAcuGAGGTT B	1803
260	AGCCAGGGAGCAUCAUUCAUUUA	1488		PSEN2:262U21 sense siNA stab04	B ccAGGGAGcAucAuucAuuTT B	1804
549	ACCGCUAUGUCUGUAGUGGGGUU	1489		PSEN2:551U21 sense siNA stab04	B cGcuAuGucuGuAGuGGGGTT B	1805
597	AAGAGCUGACCCUCAAAUACGGA	1490		PSEN2:599U21 sense siNA stab04	B GAGcuGAcccucAAAuAcGTT B	1806
730	CACGACAUUCACUGAGGACACAC	1491		PSEN2:732U21 sense siNA stab04	B cGAcAuucAcuGAGGAcAcTT B	1807
938	GUGCUCAAGACCUACAAUGUGGC	1492		PSEN2:940U21 sense siNA stab04	B GcucAAGAccuAcAAuGuGTT B	1808

947	ACCUACAAUGUGGCCAUGGACUA	1493		PSEN2:949U21 sense siNA stab04	B cuAcAAuGuGGccAuGGAcTT B	1809
2095	GAGUGUUCCCAAUGCUUUGUCCA	1494		PSEN2:2097U21 sense siNA stab04	B GuGuucccAAuGcuuuGucTT B	1810
104	UUACUGAUGAAGAAACUGAGGCC	1487		PSEN2:124L21 antisense siNA (106C) stab05	ccucAGuuucuucAucAGuTsT	1811
260	AGCCAGGGAGCAUCAUUCAUUUA	1488		PSEN2:280L21 antisense siNA (262C) stab05	AAuGAAuGAuGcucccuGGTsT	1812
549	ACCGCUAUGUCUGUAGUGGGGUU	1489		PSEN2:569L21 antisense siNA (551C) stab05	ccccAcuAcAGAcAuAGcGTsT	1813
597	AAGAGCUGACCCUCAAAUACGGA	1490		PSEN2:617L21 antisense siNA (599C) stab05	cGuAuuuGAGGGucAGcucTsT	1814
730	CACGACAUUCACUGAGGACACAC	1491		PSEN2:750L21 antisense siNA (732C) stab05	GuGuccucAGuGAAuGucGTsT	1815
938	GUGCUCAAGACCUACAAUGUGGC	1492		PSEN2:958L21 antisense siNA (940C) stab05	cAcAuuGuAGGucuuGAGcTsT	1816
947	ACCUACAAUGUGGCCAUGGACUA	1493		PSEN2:967L21 antisense siNA (949C) stab05	GuccAuGGccAcAuuGuAGTsT	1817
2095	GAGUGUUCCCAAUGCUUUGUCCA	1494		PSEN2:2115L21 antisense siNA (2097C) stab05	GAcAAAGcAuuGGGAAcAcTsT	1818
104	UUACUGAUGAAGAAACUGAGGCC	1487		PSEN2:106U21 sense siNA stab07	B AcuGAuGAAGAAAcuGAGGTT B	1819
260	AGCCAGGGAGCAUCAUUCAUUUA	1488		PSEN2:262U21 sense siNA stab07	B ccAGGGAGcAucAuucAuwTT B	1820
549	ACCGCUAUGUCUGUAGUGGGGUU	1489		PSEN2:551U21 sense siNA stab07	B cGcuAuGucuGuAGuGGGGTT B	1821
597	AAGAGCUGACCCUCAAAUACGGA	1490		PSEN2:599U21 sense siNA stab07	B GAGcuGAcccucAAAuAcGTT B	1822
730	CACGACAUUCACUGAGGACACAC	1491		PSEN2:732U21 sense siNA stab07	B cGAcAuucAcuGAGGAcAcTT B	1823
938	GUGCUCAAGACCUACAAUGUGGC	1492		PSEN2:940U21 sense siNA stab07	B GcucAAGAccuAcAAuGuGTT B	1824
947	ACCUACAAUGUGGCCAUGGACUA	1493		PSEN2:949U21 sense siNA stab07	B cuAcAAuGuGGccAuGGAcTT B	1825
2095	GAGUGUUCCCAAUGCUUUGUCCA	1494		PSEN2:2097U21 sense siNA stab07	B GuGuucccAAuGcuuuGucTT B	1826
104	UUACUGAUGAAGAAACUGAGGCC	1487		PSEN2:124L21 antisense siNA (106C) stab11	ccucAGuuucuucAucAGuTsT	1827
260	AGCCAGGGAGCAUCAUUCAUUUA	1488		PSEN2:280L21 antisense siNA (262C) stab11	AAuGAAuGAuGcucccuGGTsT	1828
549	ACCGCUAUGUCUGUAGUGGGGUU	1489		PSEN2:569L21 antisense siNA (551C) stab11	ccccAcuAcAGAcAuAGcGTsT	1829

597	AAGAGCUGACCCUCAAAUACGGA	1490		PSEN2:617L21 antisense siNA (599C) stab11	cGuAuuuGAGGGucAGcucTsT	1830
730	CACGACAUUCACUGAGGACACAC	1491		PSEN2:750L21 antisense siNA (732C) stab11	GuGuccucAGuGAAuGucGTsT	1831
938	GUGCUCAAGACCUACAAUGUGGC	1492		PSEN2:958L21 antisense siNA (940C) stab11	cAcAuuGuAGGucuuGAGcTsT	1832
947	ACCUACAAUGUGGCCAUGGACUA	1493		PSEN2:967L21 antisense siNA (949C) stab11	GuccAuGGccAcAuuGuAGTsT	1833
2095	GAGUGUUCCCAAUGCUUUGUCCA	1494		PSEN2:2115L21 antisense siNA (2097C) stab11	GAcAAAGcAuuGGGAAcAcTsT	1834
104	UUACUGAUGAAGAAACUGAGGCC	1487		PSEN2:106U21 sense siNA stab18	B AcuGAuGAAGAAAcuGAGGTT B	1835
260	AGCCAGGGAGCAUCAUUCAUUUA	1488		PSEN2:262U21 sense siNA stab18	B ccAGGGAGcAucAuucAuuTT B	1836
549	ACCGCUAUGUCUGUAGUGGGGUU	1489		PSEN2:551U21 sense siNA stab18	B cGcuAuGucuGuAGuGGGGTT B	1837
597	AAGAGCUGACCCUCAAAUACGGA	1490		PSEN2:599U21 sense siNA stab18	B GAGcuGAcccucAAAuAcGTT B	1838
730	CACGACAUUCACUGAGGACACAC	1491		PSEN2:732U21 sense siNA stab18	B cGAcAuucAcuGAGGAcAcTT B	1839
938	GUGCUCAAGACCUACAAUGUGGC	1492		PSEN2:940U21 sense siNA stab18	B GcucAAGAccuAcAAuGuGTT B	1840
947	ACCUACAAUGUGGCCAUGGACUA	1493		PSEN2:949U21 sense siNA stab18	B cuAcAAuGuGGccAuGGAcTT B	1841
2095	GAGUGUUCCCAAUGCUUUGUCCA	1494		PSEN2:2097U21 sense siNA stab18	B GuGuucccAAuGcuuuGucTT B	1842
104	UUACUGAUGAAGAAACUGAGGCC	1487	33957	PSEN2:124L21 antisense siNA (106C) stab08	ccucAGuuucuucAucAGuTsT	1843
260	AGCCAGGGAGCAUCAUUCAUUUA	1488	33958	PSEN2:280L21 antisense siNA (262C) stab08	AAuGAAuGAuGcuccuGGTsT	1844
549	ACCGCUAUGUCUGUAGUGGGGUU	1489	33959	PSEN2:569L21 antisense siNA (551C) stab08	ccccAcuAcAGAcAuAGcGTsT	1845
597	AAGAGCUGACCCUCAAAUACGGA	1490	33960	PSEN2:617L21 antisense siNA (599C) stab08	cGuAuuuGAGGGucAGcucTsT	1846
730	CACGACAUUCACUGAGGACACAC	1491	33961	PSEN2:750L21 antisense siNA (732C) stab08	GuGuccucAGuGAAuGucGTsT	1847
938	GUGCUCAAGACCUACAAUGUGGC	1492	33962	PSEN2:958L21 antisense siNA (940C) stab08	cAcAuuGuAGGucuuGAGcTsT	1848
947	ACCUACAAUGUGGCCAUGGACUA	1493	33963	PSEN2:967L21 antisense siNA (949C) stab08	GuccAuGGccAcAuuGuAGTsT	1849
2095	GAGUGUUCCCAAUGCUUUGUCCA	1494	33964	PSEN2:2115L21 antisense siNA (2097C) stab08	GAcAAAGcAuuGGGAAcAcTsT	1850

104	UUACUGAUGAAGAAACUGAGGCC	1487	33941	PSEN2:106U21 sense siNA stab09	B ACUGAUGAAGAAACUGAGGTT B	1851
260	AGCCAGGGAGCAUCAUUCAUUUA	1488	33942	PSEN2:262U21 sense siNA stab09	B CCAGGGAGCAUCAUUCAUUTT B	1852
549	ACCGCUAUGUCUGUAGUGGGGUU	1489	33943	PSEN2:551U21 sense siNA stab09	B CGCUAUGUCUGUAGUGGGGTT B	1853
597	AAGAGCUGACCCUCAAAUACGGA	1490	33944	PSEN2:599U21 sense siNA stab09	B GAGCUGACCCUCAAAUACGTT B	1854
730	CACGACAUUCACUGAGGACACAC	1491	33945	PSEN2:732U21 sense siNA stab09	B CGACAUUCACUGAGGACACTT B	1855
938	GUGCUCAAGACCUACAAUGUGGC	1492	33946	PSEN2:940U21 sense siNA stab09	B GCUCAAGACCUACAAUGUGTT B	1856
947	ACCUACAAUGUGGCCAUGGACUA	1493	33947	PSEN2:949U21 sense siNA stab09	B CUACAAUGUGGCCAUGGACTT B	1857
2095	GAGUGUUCCCAAUGCUUUGUCCA	1494	33948	PSEN2:2097U21 sense siNA stab09	B GUGUUCCCAAUGCUUUGUCTT B	1858
104	UUACUGAUGAAGAAACUGAGGCC	1487	33949	PSEN2:124L21 antisense siNA (106C) stab10	CCUCAGUUUCUUCAUCAGUTsT	1859
260	AGCCAGGGAGCAUCAUUCAUUUA	1488	33950	PSEN2:280L21 antisense siNA (262C) stab10	AAUGAAUGAUGCUCCCUGGTsT	1860
549	ACCGCUAUGUCUGUAGUGGGGUU	1489	33951	PSEN2:569L21 antisense siNA (551C) stab10	CCCCACUACAGACAUAGCGTsT	1861
597	AAGAGCUGACCCUCAAAUACGGA	1490	33952	PSEN2:617L21 antisense siNA (599C) stab10	CGUAUUUGAGGGUCAGCUCTsT	1862
730	CACGACAUUCACUGAGGACACAC	1491	33953	PSEN2:750L21 antisense siNA (732C) stab10	GUGUCCUCAGUGAAUGUCGTsT	1863
938	GUGCUCAAGACCUACAAUGUGGC	1492	33954	PSEN2:958L21 antisense siNA (940C) stab10	CACAUUGUAGGUCUUGAGCTsT	1864
947	ACCUACAAUGUGGCCAUGGACUA	1493	33955	PSEN2:967L21 antisense siNA (949C) stab10	GUCCAUGGCCACAUUGUAGTsT	1865
2095	GAGUGUUCCCAAUGCUUUGUCCA	1494	33956	PSEN2:2115L21 antisense siNA (2097C) stab10	GACAAAGCAUUGGGAACACTsT	1866
104	UUACUGAUGAAGAAACUGAGGCC	1487		PSEN2:124L21 antisense siNA (106C) stab19	ccucAGuuucuucAucAGuTT B	1867
260	AGCCAGGGAGCAUCAUUCAUUUA	1488		PSEN2:280L21 antisense siNA (262C) stab19	AAuGAAuGAuGCucccuGGTT B	1868
549	ACCGCUAUGUCUGUAGUGGGGUU	1489		PSEN2:569L21 antisense siNA (551C) stab19	ccccAcuAcAGAcAuAGcGTT B	1869
597	AAGAGCUGACCCUCAAAUACGGA	1490		PSEN2:617L21 antisense siNA (599C) stab19	cGuAuuuGAGGGucAGcucTT B	1870
730	CACGACAUUCACUGAGGACACAC	1491		PSEN2:750L21 antisense siNA (732C) stab19	GuGucccucAGuGAAuGucGTT B	1871

938	GUGCUCAAGACCUACAAUGUGGC	1492		PSEN2:958L21 antisense siNA (940C) stab19	cAcAuuGuAGGucuuGAGcTT B	1872
947	ACCUACAAUGUGGCCAUGGACUA	1493		PSEN2:967L21 antisense siNA (949C) stab19	GuccAuGGccAcAuuGuAGTT B	1873
2095	GAGUGUUCCCAAUGCUUUGUCCA	1494		PSEN2:2115L21 antisense siNA (2097C) stab19	GAcAAAGcAuuGGGAAcAcTT B	1874
104	UUACUGAUGAAGAAACUGAGGCC	1487		PSEN2:124L21 antisense siNA (106C) stab22	CCUCAGUUUCUUCAUCAGUTT B	1875
260	AGCCAGGGAGCAUCAUUCAUUUA	1488		PSEN2:280L21 antisense siNA (262C) stab22	AAUGAAUGAUGCUCCCUGGTT B	1876
549	ACCGCUAUGUCUGUAGUGGGGUU	1489		PSEN2:569L21 antisense siNA (551C) stab22	CCCCACUACAGACAUAGCGTT B	1877
597	AAGAGCUGACCCUCAAAUACGGA	1490		PSEN2:617L21 antisense siNA (599C) stab22	CGUAUUUGAGGGUCAGCUCTT B	1878
730	CACGACAUUCACUGAGGACACAC	1491		PSEN2:750L21 antisense siNA (732C) stab22	GUGUCCUCAGUGAAUGUCGTT B	1879
938	GUGCUCAAGACCUACAAUGUGGC	1492		PSEN2:958L21 antisense siNA (940C) stab22	CACAUUGUAGGUCUUGAGCTT B	1880
947	ACCUACAAUGUGGCCAUGGACUA	1493		PSEN2:967L21 antisense siNA (949C) stab22	GUCCAUGGCCACAUUGUAGTT B	1881
2095	GAGUGUUCCCAAUGCUUUGUCCA	1494		PSEN2:2115L21 antisense siNA (2097C) stab22	GACAAAGCAUUGGGAACACTT B	1882

Uppercase = ribonucleotide
u,c = 2'-deoxy-2'-fluoro U,C
T = thymidine
B = inverted deoxy abasic
s = phosphorothioate linkage
A = deoxy Adenosine
G = deoxy Guanosine
G = 2'-O-methyl Guanosine
A = 2'-O-methyl Adenosine

Table IV

Non-limiting examples of Stabilization Chemistries for chemically modified siNA constructs

Chemistry	pyrimidine	Purine	cap	p=S	Strand
"Stab 00"	Ribo	Ribo	TT at 3'-ends		S/AS
"Stab 1"	Ribo	Ribo	-	5 at 5'-end 1 at 3'-end	S/AS
"Stab 2"	Ribo	Ribo	-	All linkages	Usually AS
"Stab 3"	2'-fluoro	Ribo	-	4 at 5'-end 4 at 3'-end	Usually S
"Stab 4"	2'-fluoro	Ribo	5' and 3'-ends	-	Usually S
"Stab 5"	2'-fluoro	Ribo	-	1 at 3'-end	Usually AS
"Stab 6"	2'-O-Methyl	Ribo	5' and 3'-ends	-	Usually S
"Stab 7"	2'-fluoro	2'-deoxy	5' and 3'-ends	-	Usually S
"Stab 8"	2'-fluoro	2'-O-Methyl	-	1 at 3'-end	Usually AS
"Stab 9"	Ribo	Ribo	5' and 3'-ends	-	Usually S
"Stab 10"	Ribo	Ribo	-	1 at 3'-end	Usually AS
"Stab 11"	2'-fluoro	2'-deoxy	-	1 at 3'-end	Usually AS
"Stab 12"	2'-fluoro	LNA	5' and 3'-ends		Usually S
"Stab 13"	2'-fluoro	LNA		1 at 3'-end	Usually AS
"Stab 14"	2'-fluoro	2'-deoxy		2 at 5'-end 1 at 3'-end	Usually AS
"Stab 15"	2'-deoxy	2'-deoxy		2 at 5'-end 1 at 3'-end	Usually AS
"Stab 16	Ribo	2'-O-Methyl	5' and 3'-ends		Usually S
"Stab 17"	2'-O-Methyl	2'-O-Methyl	5' and 3'-ends		Usually S
"Stab 18"	2'-fluoro	2'-O-Methyl	5' and 3'-ends		Usually S
"Stab 19"	2'-fluoro	2'-O-Methyl	3'-end		Usually AS
"Stab 20"	2'-fluoro	2'-deoxy	3'-end		Usually AS
"Stab 21"	2'-fluoro	Ribo	3'-end		Usually AS
"Stab 22"	Ribo	Ribo	3'-end -		Usually AS

"Stab 23"	2'-fluoro*	2'-deoxy*	5' and 3'-ends		Usually S
"Stab 24"	2'-fluoro*	2'-O-Methyl*	-	1 at 3'-end	Usually AS
"Stab 25"	2'-fluoro*	2'-O-Methyl*	-	1 at 3'-end	Usually AS

CAP = any terminal cap, see for example **Figure 10**.
All Stab 00-25 chemistries can comprise 3'-terminal thymidine (TT) residues
All Stab 00-25 chemistries typically comprise about 21 nucleotides, but can vary as described herein.
S = sense strand
AS = antisense strand
*Stab 23 has single ribonucleotide adjacent to 3'-CAP
*Stab 24 has single ribonucleotide at 5'-terminus
*Stab 25 has three ribonucleotides at 5'-terminus

Table V

A. 2.5 µmol Synthesis Cycle ABI 394 Instrument

Reagent	Equivalents	Amount	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time*RNA
Phosphoramidites	6.5	163 µL	45 sec	2.5 min	7.5 min
S-Ethyl Tetrazole	23.8	238 µL	45 sec	2.5 min	7.5 min
Acetic Anhydride	100	233 µL	5 sec	5 sec	5 sec
N-Methyl Imidazole	186	233 µL	5 sec	5 sec	5 sec
TCA	176	2.3 mL	21 sec	21 sec	21 sec
Iodine	11.2	1.7 mL	45 sec	45 sec	45 sec
Beaucage	12.9	645 µL	100 sec	300 sec	300 sec
Acetonitrile	NA	6.67 mL	NA	NA	NA

B. 0.2 µmol Synthesis Cycle ABI 394 Instrument

Reagent	Equivalents	Amount	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time*RNA
Phosphoramidites	15	31 µL	45 sec	233 sec	465 sec
S-Ethyl Tetrazole	38.7	31 µL	45 sec	233 min	465 sec
Acetic Anhydride	655	124 µL	5 sec	5 sec	5 sec
N-Methyl Imidazole	1245	124 µL	5 sec	5 sec	5 sec
TCA	700	732 µL	10 sec	10 sec	10 sec
Iodine	20.6	244 µL	15 sec	15 sec	15 sec
Beaucage	7.7	232 µL	100 sec	300 sec	300 sec
Acetonitrile	NA	2.64 mL	NA	NA	NA

C. 0.2 µmol Synthesis Cycle 96 well Instrument

Reagent	Equivalents:DNA/ 2'-O-methyl/Ribo	Amount: DNA/2'-O-methyl/Ribo	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time* Ribo
Phosphoramidites	22/33/66	40/60/120 µL	60 sec	180 sec	360sec
S-Ethyl Tetrazole	70/105/210	40/60/120 µL	60 sec	180 min	360 sec
Acetic Anhydride	265/265/265	50/50/50 µL	10 sec	10 sec	10 sec
N-Methyl Imidazole	502/502/502	50/50/50 µL	10 sec	10 sec	10 sec
TCA	238/475/475	250/500/500 µL	15 sec	15 sec	15 sec
Iodine	6.8/6.8/6.8	80/80/80 µL	30 sec	30 sec	30 sec
Beaucage	34/51/51	80/120/120	100 sec	200 sec	200 sec
Acetonitrile	NA	1150/1150/1150 µL	NA	NA	NA

- Wait time does not include contact time during delivery.
- Tandem synthesis utilizes double coupling of linker molecule